SAFETY AND PHARMACOLOGICAL PROFILE OF *PATTAI VALLATHAGI*

The dissertation Submitted by

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CONTENTS

INTRODUCTION

1. INTRODUCTION

The Siddha system dates back to 5000 B.C.The 18siddhars headed by Saint Agathiyar had established the system.Lord siva, Lord Murugan, Saint Agasthiyar hare symbalised the tamil literature which is inseparable from siddha medicine and tamil tradition.

The current research was on ***Pattai Vallathagi*** to evaluate the safety and pharmacological activity in animal models.

The drug *Pattai vallathagi* was indicated for Kuttam, Kiranthi,Kodiya Viranangal which was selected from the siddha literature***"Siddha anuboga vaithiya navaneetha thirattu (part 10)"*** First edition-2010, pg.no:1581 authored by ***Hakkim P. Mohammed Abdulla sayub.***

The literary evidence of the drug *Pattai Vallathagi* strongly support that it possesses anti-inflammatory,analgesic and anti-histamine activities for that purpose. It has been selected for this study.

All the ingredients were identified and authenticated by the experts.The test drug was prepared by the given procedure.

The chemical analysis was done at Bio-chemistry lab, NIS. The Chemical analysis of the drug *Pattai Vallathagi* revealed the presence of Sulphate,Calcium, Iron, Potassium,Alkaloid, Tannin, which provided the key ingredients present in the drug thus it accounts the efficacy of the drug.

The ICP-OES and HR SEM analysis was done at SAIF, IIT Madras. The ICP-OES study revealed that the heavy metals like As, Pb, Cd were found below detection limit in the test drug. Calcium,Potassium,Phosphorus,Sodium,Sulphur were present

In HR SEM analysis the particle size of *Pattai Vallathagi* was 1.2-2.4µ

Preclinical evaluation of acute and sub-acute toxicity study was carried out in K.K College of Pharmacy, Gerugambakkam and sub-chronic toxicity study of the drug was carried out in animal house, NIS, Chennai.

Wistar albino rats of either sex of weight 150-200 gm were used for toxicity and pharmacological studies. The animals were kept under standard conditions 12:12(day/night cycles) at room temperature in polypropylene cages. The animals were fed on standard pelleted diet and Potable water in polypropylene bottles *ad libitum*. The

animals were housed for one week prior to the experiments to acclimate to animal house conditions.

In acute oral toxicity study, various dose level of *Pattai Vallathagi* 5, 50, 300 and 2000 mg/kg b.w. was mixed with water and was administered to female wistar albino rats which showed no abnormalities in external observation and necropsy examination and all the vital organs were normal.

In Repeated dose 28 days oral toxicity study and Repeated dose 90 days oral toxicity study , various doses level of *Pattai Vallathagi* 900 mg/kg and1800 mg/kg was mixed with water and administered orally, did not show any significant changes in hematological parameters and histopathological slides of various organs.

The Pharmacological study anti -inflammatory, analgesic and anti -histamine activity of the drug was carried out in wistar albino rat, swiss albino mice and guinea pig as per OECD guideline in K.K College of Pharmacy, Gergambakkam revealed that the drug *Pattai Vallathagi* exhibited significant anti inflammatory, analgesic.and anti histamine activity

The above studies showed that the drug *Pattai Vallathagi* was safe in animal models and may be tried for further studies to establish the clinical use.

AIM AND OBJECTIVE

2. AIM AND OBJECTIVES

AIM

The aim of the study is to evaluate the Safety and Pharmacological profile of the test drug "***PATTAI VALLATHAGI***'' in animal models .

OBJECTIVE

The following methodology was adopted to evaluate the safety and pharmacological activities of the test drug.

- Review of various information (Siddha and modern) relevant to the study.
- Preparation of the drug as per classical Siddha literature.
- Analytical study of the prepared drug
    - Physico chemical and phytochemical analysis
    - Chemical analysis to evaluate acidic and basic radicals.
    - Heavy metal analysis
    - Elemental analysis
    - Analysis of Particle size

- Screening the toxicity studys in animal models
    - Acute oral toxicity study (OECD – 423 Guideline)
    - Repeated dose 28 days oral toxicity study (OECD – 407 Guideline)
    - Repeated dose 90 days oral toxicity study (OECD – 408 Guideline)

- Evaluation of pharmacological activities in Wister albino rat and mice and guinea pig
    - Anti- inflammatory (Cotton pellet induced granuloma method)
    - Analgesic (Eddy's hot plate method)
    - Anti-histamine (ileum cut terminal method)

MATERIALS AND METHODS

3. MATERIALS AND METHODS

STANDARD OPERATIVE PROCEDURE OF *PATTAI VALLATHAGI*

Drug selection :

To evaluate the efficacy of ***Pattai Vallathagi*** has been selected as per siddha literature ***Siddha anuboga vaithiya navaneetha thirattu*** (part -10),pg.no: 1581 written by ***Hakkim P.Mohammed Abdulla sayub***

Ingredients[1]

- ***Purified cherangkottai*** *(Semecarpus anacardium)* - *2 palam (70gms)*
- ***Purified Sesamum Seeds*** *(Sesamum indicum)* - *8 palam (280gms)*
- ***Purified Nellikkai ganthagam*** *(Purified Sulphur)* - *2palam (70gms)*
- ***Purified Turmeric*** *(Curcuma longa)* - *2 palam (70gms)*
- ***Purified Parangi Pattai*** *(Smilax chinensis)* - *4 palam (140gms)*
- ***Ellennai*** - *8 palam (280gms)*
- ***Purified Panai vellam*** - *6 palam (210gms)*

Source of collection :

The druge were purchased from authorized country raw drug store in Chennai.Sulphur was purified and the medicine was prepared in the Gunapadam laboratory of National Institute of Siddha

Identification and Authentication of the drug:

Mineral drug was authenticated by the chemist in Central Research Institute of Siddha, Arumbakkam,Chennai All the plant meterials were identified and authenticated by the Botanist, National Institute Of Siddha, Tambaram Sanatorium, Chennai

Purification of the ingredients :

Purification of *Ganthagam*[5]

Ganthagam crushed into small pieces and it was put into an iron vessel and added butter into the vessels and heated until the sulphur was melted completely and it was transferred into other vessel which contains cow's milk.After cooling it was washed with

tap water.This process was repeated for 30 times.In every time the fresh butter and milk were used.

Purification of *cherangkottai*[(2)]

Take *cherangkottai* and tamarind leaf in the ratio of 1:3and place it in a mud vessel,add water equivalent to eight times the amount of mixture and than boil it till it is reduced to 1/8 of the initial quantity. Then dry it in sunlight. The procedure is repeated for seven times

Purification of *parangichakkai* [(3)]

Dried tuber of Smilax china is steamed using milk, dried well and powdered.

Purification of Sesamum seeds [(3)]

Sesamum seeds are fried well.

Purification of Turmeric[(3)]

Outer peel is scrapped and dried in sunlight.

Method of drug preparation [(1)]

Cherangkottai was placed in mortar and sesame seeds are added little by little and was crushed with pestle. Then palm jaggery was added and crushed till content become electuary form. Then the power of remaining ingredients are added and crushed.The above contentswas mixed with gingelly oil and preserved in porcelain dish.

Drug labelling :

Name	:	*Pattai Vallathagi*
Color	:	Dark brown
Therapeutic Dose	:	1gm ,Twice a day
Adjuvent/vehicle	:	Water
Date of preparation	:	20.7.2015
Date of expiry	:	1 years from the date of manufacture
Indications	:	*Kuttam, Kiranthi,Kodiya viranangal.*

INGREDIENTS OF *PATTAI VALLATHAGI*

BEFORE PURIFICATION

Semecarpus anacardium

Smilax china

Curcuma longa

AFTER PURIFICATION

Cherangkottai

Parangi pattai

Manjal

Sulphur

Ganthagam

Sesamum indicum -Ell

Palm jaggery-Panai vellam

Gingelly - Oil

PATTAI VALLATHAGI

REVIEW OF LITERATURE

GUNAPADAM REVIEW

4. REVIEW OF LITERATURE

4.1 GUNAPADAM REVIEW

பறங்கிப் பட்டை – Parangi pattai

Botanical Name : *Smilax chinensis.Linn*

Family : Liliaceae

English Name : China root

வேறு பெயர்கள்[4]

மதுஸ்மிகம்,

மதுஸ்மீகி,

பறங்கிச்சக்கை,

சீனப்பட்டை.

பயன்படும் உறுப்பு:

கிழங்கு.

Organoleptic characters

- **சுவை** : இனிப்பு
- **தன்மை** : தட்பம்
- **பிரிவு** : இனிப்பு

செய்கை:

மேகப்பிணிவிலக்கி

உடற்றேற்றி

தூய்மையாக்கி

பொதுகுணம்:

தாகம் பலவாதந் தாதுநட்டம் புண்பிளவை

மேகங் கடிகிரந்தி வீழ்மூலந் -தேகமுடன்

குட்ட பகந்தரமேற் கொள்வமனம் போம்பறங்கிப்

பட்டையினை யுச்சரித்துப் பார்.

பொருள் :

இதனால், நீர்வேட்கை, பற்பல வளிநோய், புண், பிளவை, நீரிழிவு, கடிவிடம், சிரங்கு, மூலமுளை, முடவாதம், குறைநோய், ஐயம், மகரந்தப்புண், வாந்தி இவை நீங்கும். ஆண்மை உண்டாம்.

பறங்கிப்பட்டை சேரும் பிறமருந்துகள் :

1.மேக சிந்தாமணி மெழுகு

2.பறங்கிப்பட்டை பதங்கம்

3.சர்வ மேகத்தெண்ணெய்

4.மேகநாத தைலம்

5.பறங்கிப்பட்டை இரசாயனம்

6.இரச மெழுகு

சேராங்கொட்டை -Cherangkottai

Botanical Name : *Semecarpus anacardium.Linn*

Family : Anacardiaceae

English Name : Marking Nut Tree

வேறு பெயர்கள்[4] :

வல்லாதகி,

வல்லாதி,

பல்லாதகி,

எரிமுகி,

நந்திவித்து,

கிட்டாக்கனி கொட்டை.

பயன்படும் உறுப்பு:

கொட்டை பருப்பு.

Organoleptic characters

- **சுவை** : கைப்பு, விறுவிறுப்பு
- **தன்மை** : வெப்பம்
- **பிரிவு** : கார்ப்பு

செய்கை :

உடற்றேற்றி

பொதுகுணம் :

குட்டங் கயரோகங் கொல்லும் விடபாகந்
துட்டந் தருகிருமி சூலையும் போம் - மட்டலருங்
கூந்தன்மயி லேகிரந்திக் கூட்டம் போஞ் செங்கையில்
ஏந்து சேங் கொட்டைதனை யே.

-

பொருள் :

இது பெருநோய், இளைப்பு நோய், நஞ்சுகள், சூலை, திமிர்ப்படை, கருப்புப்படை, வெண்படை, தீராக்கடி, மூலம், வளிநோய்கள், குன்மம் இவைகளை போக்கும்.

சுருங்கச் சொல்லின் யாவரும் மெச்சும் இரசத்தால் தீரும் பெருநோய்களும், சேராங்கொட்டையால் தீரும்

சேராங்கொட்டை சேரும் பிறமருந்துகள் :

1.இடிவல்லாதி இளகம்

2. ஆனந்த வினோதச் சூரணம்

3.நந்தி மை :

மஞ்சள் - **Manjal**

Botanical Name : *Curcuma longa.Linn*

Family : Zingiberaceae

English Name : Turmeric

வேறு பெயர்கள்[4]

நிசி,

பிதம்,

அரிசனம்,

கான்சானி.

பயன்படும் உறுப்பு :

கிழங்கு.

Organoleptic characters

- **சுவை** : கார்ப்பு கைப்பு
- **தன்மை** : வெப்பம்
- **பிரிவு** : கார்ப்பு.

செய்கை:

மணமூட்டி

அகட்டுவாய்வகற்றி

ஈரல்தேற்றி

பொதுக்குணம்:

பொன்னிறமாம் மேனி புலானாற்ற மும்போகும்
மன்னு புருட வசியமாம் -பின்னியெழும்
வாதபித்த தோடமையம் வாதம்போந் தீபனமாங்
கூர்ந்தமஞ்ச ளின்கிழங்குக்கு.

பொருள் :

இதனால், வாந்தி, வளி, தீ, ஐயக்குற்றம், தலைவலி, நீரேற்றம், வெள்ளை, மூக்கு நீர்பாய்தல், ஐவகைவலி, வீக்கம், வண்டுகடி, பெரும்புண் இவை போம்.

4. *பனை வெல்லம்* – Palm jaggary

பொதுகுணம்[4]

‘‘----------தங்குபனை

வெல்லத்தால் வாதபித்தம் வீறுகபஞ் சன்னிநோய்

வல்லருசி குன்மமறு மால்’’

-அகத்தியர் குணவாகடம்

பனை வெல்லத்தால் முக்குற்றத்தால் வரும் நோய்களும்,முப்பிணி,சுவையின்மை, குன்மம் இவைகளும் நீங்கும்.

எள் - **Ell**

Botanical Name : *Sesamum indicum,Linn*

Family **:** Pedaliaceae

English Name : Gingeli oil plant,Gingelly, sesame

வேறு பெயர்கள்[4] **:**

திலம்

பயன்படும் உறுப்பு:

இலை, பூ,காய்,விதை.

Organoleptic Characters

- **சுவை** : இனிப்பு
- **தன்மை** : வெப்பம்
- **பிரிவு** : இனிப்பு

செய்கை :

உரமாக்கி

சிறுநீர்பெருக்கி

மலமிளக்கி

பொதுகுணம் :

‘‘எள்ளுமருந் தைக்கெடுக்கும் எறனலாந் திண்மைதரும்

உள்ளிலையைச் சேர்க்கும் உதிரத்தைத் - தள்ளுமிரு

கண்ணுக் கொளிகொடுக்குங் காசமுண்டாம் பித்தமுமாம்

பண்ணுக் கிடர்புரியும் பார்’’

எள் நெய் (நல்லெண்ணெய்) - **Sesamum oil**

எள்ளை ஆட்டி எடுக்கும் எண்ணெய் நல்லெண்ணெய்.

செய்கை[4]

உள்ளழலாற்றி

மலமிளக்கி

உடலுரமாக்கி

வறட்சியகற்றி.

பொதுகுணம் :

புத்திநயனக் குளிர்ச்சி பூரிப்பு மெய்ப்புளகஞ்

சத்துவங் கந்தி தனியிளமை - மெத்தவுண்டாங்

கண்ணோய் செவிநோய் கபாலவழல் காசநோய்

புண்ணோய்போ மெண்ணெய்யாற் போற்று.

பொருள் :

நல்லெண்ணெயில் இரண்டு அல்லது நாலு உச்சிக்கரண்டியளவு ஒவ்வொருநாளும் கொடுத்துக் கொண்டுவர உடல்பூரிக்கும்.தினவு,படை,சொறி சிரங்கு நீங்கி தோல் மெலியும்.

கந்தகம் -Ganthagam

வேறு பெயர்கள்[5]

- பீஜம்
- சக்தி,
- நாதம்,
- நாற்றம்,
- பொன்வர்ணி,
- அதீதப்பிரகாசம்,
- செந்தூரத்தாதி,
- இரசசுரோணிதம்.

பதார்த்த குண சிந்தாமணியில் நெல்லிக்காய் கந்தகம்,வாண கந்தகம் என்று 2 வகைகளின் குணங்கள் கூறப்பட்டுள்ளது.மருந்துகளில் கையாளப்படுவது நெல்லிக்காய் கந்தகம்தான்.

கிடைக்கும் இடங்கள் :

- நேபாளம், காஷ்மிரம், ஆப்கானிஸ்தானம், பர்மா முதலிய இடங்களில் கந்தகம் கிடைக்கிறது.

சிறப்பு குணங்கள் :

- இதனுடைய பெயரே இதற்கு மணமுண்டென்பதை விளக்கம்.
- தாது, தாவர பொருள்களிலும் கலப்புற்று இருக்கும்,

Organoleptic characters

- **சுவை** : கைப்பு, துவர்ப்பு.
- **செய்கைகள்** : மலமிளக்கி,
  உடல் தேற்றி,
  வியர்வை பெருக்கி,
  கிருமி நாசினி.

கந்தகம் விரேகியில் சிறப்பாக செயல்பட்டு சுரப்பை அதிகப்படுத்தும். தவிர இது தோல், அசுகங்களில் சளிச்சவ்வினுள்ள சுரப்பையும் அதிகரிக்கும்.

நெல்லிக்காய்க் கந்திக்கு நீள்பதிணெண் குட்டமந்தம்
வல்லை கவிசைகுன்ம வாயுகண்ணோய் - பொல்லா
விடக்கடிவன் மேகநோய் வீறுசுரம் பேதி
திடக்கிரக ணீகபம்போந் தேர்.

பொருள் :

நெல்லிக்காய்க் கந்தியினால் பதினெண் குட்டம், மந்தம், கல்லீரல் வீக்கம், வாதசுரம், பெருவயிறு, குன்ம வாயு முதலியவை தீரும்.பெருவயிறுகளில் ஒன்றாகிய கவிசை, வாத சுரம் போகும்.

"மாதர் மகவை வளர்ப்பதுபோ லேயுடம்பை
யாதரவா கத்தேற்றி யாக்கையினால் - மீதாக
மேவி யடர்நோயின் வெப்பத்தை மாற்றுதலாற்
றேவியுர மென்பதுடல் தேர்."

கந்தகம், தாய் மகவை வளர்ப்பது போல நோய்களின் வெப்பத்தை மாற்றி உடம்பைத் தேற்றுவிக்கும்.

கந்தகம் சேரும் பிறமருந்துமள் :

1.கந்தக பற்பம்
2.பஞ்சமுக செந்தூரம்
3.கந்தக லேகியம்
4.கந்தக தைலம்
5.கந்தக ரசாயணம்
6.ஆறுமுகச் செந்தூரம்
7.இரச பற்பம்
8.மகா வசந்த குசுமாகாம்
9.ஆனந்த வினோத சூரணம்

BOTANICAL REVIEW

4.2. BOTANICAL REVIEW

Smilax china _ *Parangi pattai*

Synonym - ***Smilax chinensis***[7]

Family - Smilacaceae

Common names:

Catbriers, greenbriers, prickly- ivys and smilaxes

Vernacular names

Tamil - Parangichekkai

English - China root

Parts used

Tuber

Organoleptic characters

Taste - Sweet

Nature - Coolant

Division - Sweet

Action

Depurative, Antisyphilitic

Chemical components

Tubers - Tannin, Steroidal saponin Diosgenin, a steroidalsapogenin, is reported from *S. menispermoidea*. Other active compounds reported from various greenbrier species are Parillin (also sarsaparillin or smilacin), sarsapic acid, sarsapogenin, sarsaponin

Pharmacological activity

Anti inflammatory, Antioxidant, Anticonvulsant.

Article related to the research :

Acute toxicity study[10]

The methanolic extract and isolated flavonoid quercetin from the rhizome of *S.china* were found to be safe in the doses used.No abnormality in the gross behavioral studies and no mortality were noted in all the tested doses.

Pharmocological Activity[11]

The methanolic extract of *Smilax chinensis* in doses of 250mg/kg and 400mg/kg revealed significant anti inflammatory and analgesic activity.These results suggest that the ethyl acetate and methanolic extracts of *Smilax chinensis* Linn possesses analgesic and anti inflammatory activities

Medicinal uses[8]

Tubers are used to treat venereal diseases, rheumatic disorders and chronic skin affections.

Curcuma longa – Manjal

Synonym - *Curcuma domestica Val.*[7]

Family - Zingiberaceae

Vernacular name

Tamil - Manjal, nisi, peetham

Hindi - Haldi

English - Turmeric

Parts used

Rhizome

Organoleptic characters

Taste - Acrid, Bitter

Nature - Hot

Division - Acrid

Action

Rhizome - Antiseptic, anthelmintic, carminative, aromatic.

Chemical constituents

Major

Curcuminoids , the yellow colouring principles of which curcumin constitutes 50-60%. Curcumin , the major component is a well known anti- inflammatory agent.

Minor

Desmethoxycurcumin, Bisdesmethoxycurcumin, dihydrocurcumin, Ukonan A, B, C, D

The best-studied compound is curcumin, which constitutes 3.14% (on average) of powdered turmeric. In addition, other important volatile oils include turmerone, atlantone, and zingiberene.

Some general constituents are sugars, proteins, and resins.

Pharmacological activity

Anti – inflammatory.

Article related the research :

Pharmocological activity[12]

The related article revealed that the *Curcuma longa* aqueous drops definitely delayed healing of superficial corneal wounds (P less than 0.001),delayed healing of penetrating corneal wounds also and markedly reduced the tensile strength of corneal wounds.

Medicinal uses[8]

A fresh juice is commonly used in many skin conditions, including eczema, chicken pox, shingles, allergy, and scabies.

In India, turmeric has been used as a remedy for stomach and liver ailments, as well as topically to heal sores, basically for its supposed antimicrobial property.

The turmeric powder is applied over the cut with profuse bleeding in any part of the body.

It is mixed with gingelly oil and applied to the body to preent skin eruptions

The fumes of turmeric is used to relieve hysterical fits

In Catarrh and coryza the inhalation of the fumes of the burninf turmeric from the nostrils causes a copious mucous discharge and gives instant relief.

Semecarpous anacardium - cherangkottai

Family : Anacardiaceae[7]

Vernacular names

Tamil	-	Serangkottai
English	-	Marking nut
Hindi	-	Bhela

Parts used:

Fruit(seeds),

Organoleptic characters

Taste	-	Bitter
Nature	-	Hot
Division	-	Acrid

Action

Kernal	-	Carminative, cardiac tonic
Oil	-	Antiseptic, cholagogue

Chemical Constituents:

Anacordic acid, Cardol, Riboflavin, Thiamine, Histidine, Isoleucine, leucine, Bhilawanol, Biflavanoids A, B, C, Oleic acid, Palmitic acid,

Article related the research :

Pharmocological activity [13]

The related article revealed that the extract of *Semicarpus anacardium* indicated the potent anti inflammatory effect and therapeutic efficacy of *Semecarpus anacardium LINN*.nut extract against all phases of inflammation, is comparable to that of indomethacin.

Medicinal uses[8]

The extract of the fruit is effective against human epidermoid carcinoma of the nasopharynx .

Ripe fruits are regarded as stimulant, digestive, nervine

It is a good cardiac tonic and a general respiratory stimulant

Oil is used externally in gout, leprosy and leucoderma.

Exudate from the bark is useful in nervous debility and in leprous and venereal affections.

It is a powerful emmenagogue and producs good effect in dysmenorrhoea amenorrhoea, pelvic cellulitis and peritonitis.

Sesamum indicum - Ell

Synonym - *Sesamum orientale L.*[7]

Family - Pedaliaceae

Vernacular names

Tamil - Ell

Hindi - Thil

English - Sesame

Parts used

Seeds

Action

Seeds - diuretic, laxative, demulcent.

Variety

Three varieties of sesamum seeds are found; black,white, red or brown. The black variety is the most common and yields the best quality of oil and is best suited for medicinal purposes.

Organoleptic character

Taste - Sweet

Character - Hot

Division - Sweet

Chemical Constituents

Sesame seeds contain the lignans, sesamolin, sesamin, pinoresinol and lariciresinol, histidine, tryptophan, tyrosine, valine, ascorbic acid, biotin, folic acid, niacin, neutral lipids.

Pharmacological activity:

Wound healing, Antitumour**,** Antibacterial**,** Antioxidant

Medicinal uses[8]

A plaster made from the seeds is applied to burns and scalds.

Borassus Flabellifer - Panai

Family name - Arecaceae[7]

English - Palmyra

Palm Jaggery[29]

Color : Off white top pale yellowish white.

Preparation : It is prepared by boiling the sap of palmyra palm.

Physical state : Amorphous Solid.

Palm jaggery is an extract derived from the palm trees.palm jaggery is an excellent substitute for white sugar.It's an nutritious sweetenes.Palm jaggery is reported to have more nutritional and medicinal value than cane sugar.It's a good source of Vitamine B complex,and it also contain ascorbic acid.It is delicious and has mineral salts too.

Palm jaggery benefit for health are :

Rich source of minerals :

Palm jaggery is rich in essential minerals.According to some studies it has 60 times more minerals than white sugar.

Rich in nutrients :

Palm jaggery is rich in iron.It's regular consumption increase hemoglobin level treats anemia. palm jaggery contain essential nutrients like, Magnesium, Potassium.Megnesium is vital for the proper functioning of the nervas systems and the Pattassium regulates the blood pressure and the heart function.

Active cleanse:

Palm jaggery also cleans up your system.It cleanses the respiratory tract,lung, stomach.

Relieves constipation :

Palm jaggery is full of dietary fibers.These fibers help to treat constipation and indigestion.It also stimulates bowel movement.

Sesame oil (Gingelly oil)

Sesame oil is an edible vegetable oil derived from sesame seeds.It's also known as til oil.Besides being used as a cooking oil in south India.

Composition[30]

Sesame oil is composed of the following fatty acids :

Linoleic acid (41% of total),

Oleic acid (39%),

Palmitic acid (8%),

Stearic acid (5%) and other in small amounts

Nutritional value per 100 g

Energy	3,699kJ (884 k cal)
Carbohydrates	0.00g
Fat	100.00g
Saturated	14.200g
Monounsaturted	39.700g
Polyunsaturated	41.700g
Vitamine E	(9%) 1.40mg
Vitamine K	(13%)13.6µg

Medicinal use

The mixture of gingili oil and yellow of the egg is a good ointment for burns, itching etc. When there is burning sensation in the eyes, apply gingili oil over the thumbs of the foot. The oil is applied for cutaneous lesions of leprosy. The oil is given internally for gonorrhea

MINERALOGICAL REVIEW

4.3 MINERALOGICAL REVIEW

Sulphur[9]

It is a non metallic element found free in beds of gypsum and in a state of sublimation in regions of extinct volcanoes and in combination with several ores called pyrites as sulphates and sulphides of iron, copper, lead, zinc, mercury.

Sulphur is a chemical element with symbol **S** and atomic number 16. It is an abundant, multivalentnon-metal. Elemental sulfur is a bright yellow crystalline solid when at room temperature.

Synonyms

Sulphur, Brime stone, Colloidal sulphur, floor of sulphur

Distribution

It occurs in Nepal, Kashmir, Afghanistan and Burma.

Physical characters

- Colour is a strong yellow colour in thick crystals to pale yellow in massive or powdery forms.
- Luster is vitreous to more often resinous or earthy in massive forms
- Transparency is transparent to translucent
- Crystal system is orthorhombic
- Crystal habits include mostly massive or powdery forms but well shaped blocky crystals are common.
- Cleavage is very poor
- Fracture is conchoidal
- Streak is yellow
- Hardness is 2
- Specific gravity is 2.0 – 2.1
- Best Field Indicators are colour, odour, heat sensitivity, lack of good cleavage and crystal habit

Chemical Properties

Atomic number	-	16
Atomic mass	-	32.06g.mol^{-1}
Density	-	2.07g.cm^{-3} at 20ºc
Melting point	-	113ºc
Boiling point	-	445ºC
Isotopes	-	5
Energy of first ionization	-	999.3 kJ.mol^{-1}
Energy of second ionization	-	2252 kJ.mol^{-1}
Energy of third ionization	-	3357 kJ.mol^{-1}
Standard potential	-	-0.51 V

Sources

Sulphur is an essential element for all life, and is widely used in biochemical processes. In metabolic reactions, sulphur compounds serve as both fuels (electron donors) and respiratory (oxygen-alternative) materials (electron acceptors).

The vast majority is produced as a by- product of oil refining and natural gas processing. Sulphur is readily available in protein foods- eats, fish, poultry, eggs, milk and legumes. Complete vegetarians and people on low- protein diets may not get sufficient amounts of sulphur.

Many sulphur compounds are odoriferous, and the smell of odorized natural gas, skunk scent, grapefruit, and garlic is due to sulfur compounds. Hydrogen sulfide produced by living organisms imparts the characteristic odour to rotting eggs and other biological processes.

Sulphur is an important part of many enzymes and in antioxidant molecules like glutathione and thioredoxin. Organically bonded sulphur is a component of all proteins, as the amino acidscysteine and methionine.

Disulfide bonds are largely responsible for the mechanical strength and insolubility of the protein keratin, found in outer skin, hair, and feathers, and the element contributes to their pungent odour when burned.

Uses

It is used in scabies.

Joined problems may helped by chondrotin sulphate, which is found high amounts in the joint, which is found high in the joint tissues.

Magnesium sulphate which is not absorbed is used as a laxative.

It is used for treatment of Itchy skin, Psoriasis, Migrane headaches, Indigestion, Haemorrhoids, Acne, Eczema, Painful and irregular menstruation.

ANALYTICAL STUDY OF *PATTAI VALLATHAGI*

5. ANALYTICAL STUDY OF THE DRUG *PATTAI VALLATHAGI*

Analytical study brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Analytical study includes many studies such as its organoleptic character physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug. Thus Analytical study brings the efficacy and potency of the drug.

As per AYUSH protocol for , Analytical study the following parameters were evaluated.

Analytical study of the drug includes:

- **Organoleptic characters**
  - Colour
  - Oder
  - Taste
  - Texture

- **Physicochemical analysis**
  - Determination of Ash Values
  - Physical characterization

- **Chemical analysis**
  - Preliminary Basic and Acidic radical studies

- **Phytochemical analysis**

  HPTLC and TLC

- **Heavy metal analysis**
- **Microbial analysis**
- **Elemental analysis**

Inductively Coupled Plasma Optical Emissios Spectrometry(ICP- OES)

- **Analysis of particle size**

Scanned Electron Microscopy (HR-SEM)

PHYSICO CHEMICAL ANALYSIS

5.1 Organoleptic characterization of *Pattai vallathagi*:

Colour

The medicine was taken into watch glasses and placed against white back ground in white tube light. It was observed for its colour by naked eye.

Odour

The medicine was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling.

The results of organoleptic characterization were showed in table (1)

5.2 Physicochemical Analysis of *Pattai Vallathagi*

Physical properties of *Pattai vallathagi*

The physical properties of *Pattai vallathagi* was analyzed at Captain Srinivasa murti Reseach Institute of Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-106.

pH at 10% of aqueous solution:

Five grams of *Pattai vallathagi* was weighed accurately and placed in clear 100 ml beaker. Then 50 ml of distilled water was added to it and dissolved well. After 30 minutes it was then applied in to pH meter at standard buffer solution of 4.0, 7.0, and 9.2.

Determination of Ash Values:

1. Total Ash

3gm is accurately weighed and incinerated in a crucible dish at a temperature not exceed 450^{o}C until free from carbon. It is then cooled and weighed. The % w/w of ash with reference to the air-dried powder is calculated.

2. Acid insoluble Ash

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml 10% Hcl. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450^{o}C. The silica crucible and residue are weighed until constant weight is attained.

3. Loss on Drying

The drug *Pattai vallathagi* was dried in the oven at 100- 105°C to constant weight.

4. Determination Of Specific Gravity :

Clean a specific gravity bottle by shaking with acetone and then with ether.Dry the bottle and note the weight.Cool the sample solution to room temperature.Carefully fill the specific gravity bottle with the test liquid and insert the stopper and remove the surplus liquid and note the weight.Repeat the procedure using distilled water in place of sample solution.

Calculation:

$$\text{Specific gravity} = \frac{\text{weight of test sample held in specific gravity bottle}}{\text{Weight of water held in specific gravity bottle}}$$

5. Determination of Fat Content :

Accurately weight 3-4g of sample into a 500ml beaker.Add slowly while stirring 45ml of boiling water to give a homogenous suspension.Add 55ml of appr.8 M Hydrochloric acid.Cover with watch glass boil gently for 15 minutes.Rinse the watch glass with 100ml of water. Filter through Whatman no.42 and continue the washing till the filtrate is chloride free. Transfer wet paper and residue to defatted extraction thimble and dry in a small beaker at 100˚ C in hot air over.place thimble in Soxhlet.Rinse digestion beaker, drying beaker and watch glass with three 50ml portions of Petroleum ether and add washings to thimble.Reflux digested residue for 4 hours.Remove flask and evaporate petroleum ether (B.P 40-60˚C) on steam bath.Dry flask at 100-101˚C to constant weight.Cool in a desiccator to room temperature and weight until constant weight is attained.Do the duplicate determination.

$$\textbf{Calculation} = \frac{\text{Weight of petroleum ether extract} \times 100}{\text{Weight of the sample taken}}$$

6. Determination of sugar content (Lane Eynon's method)

Procedure :

Preparation of Fehling's solution : It is prepared by mixing equal volumes of solutions A and B.

Solution A : Dissolve 34.639g of pure crystallized $CuSO_4.5\ H_2O$ in water and make up to 500ml

Solution B: Dissolve 173 g of Rochelle salt (Potassium Sodium tartrate, $KNaC_4H_4O_6.4H_2O$),50g of Sodium hydroxide in water and make up to 500ml Mix equal volumes of A and B just when needed.

Dissolve 1 g of Methylene blue in 100ml water.

Methylene blue indicator : Dissolve 1g of Methylene blue in 100ml of water.

Sample preparation :

Take 10 ml/10 g of sample in a 250 ml volumetric flask and add 200 ml water, add slight excess solid basic Lead acetate to remove tannins and without disturbing the solution make up to the mark.Shake and filter.Add slight excess of solid Sodium oxalate to remove excess of basic Lead acetate.This filtrate is used for the estimation of reducing sugar.

6.1 Reducing sugar : Take the suger solution in a 50 ml burette

Preliminary titration

Pipette 10 ml of Fehling ‘s solution into a 250 ml conical flask, and add from the burette,15 ml of the sugar solution.Boil the liquid on asbestos-covered gauze and add further quantities of the sugar solution (One ml at a time) at 10 to 15 second intervals to the boiling liquid until the blue colour is nearly discharged.Then add 3-5 drops of aqueous Methylene blue solution (1%) and continue the titration until the indicator is completely decolourised.

Accurate titration

Repeat the titration, adding before heating, almost all of the sugar solution required to effect reduction of copper.Boil gently for two minutes,add 3-5 drops of Methylene blue indicator and complete the titration within a total boiling time of three minutes.At the end point all the blue colour should be discharged and the liquid should be red.The proportions of the various sugars,equivalent to 10 ml of Fehling’s solution are given in the table.

6.2 Total sugar

Take 20 ml of reducing sugar solution,and add 10 ml of concentrated Hydrochloric acid and keep it aside overnight.Neutralise with approximately 1M Sodium hydroxide and make up to 100 ml in a volumetric flask.Determine the total sugar content by the titrimetric method described above.

Repeat the experiment twice and take the average value.

Calculation

$$\text{mg of sugar in 100 ml} = \frac{\text{Total reducing sugar from table x 100}}{\text{Titre}}$$

$$\text{Reducing sugar \%} = \frac{\text{mg of sugar in 100 ml x 250 x 100}}{1000 \times 100 \times 10}$$

$$\text{Total sugar \%} = \frac{\text{mg of sugar in 100 ml x 250 x 100 x100}}{1000 \times 100 \times 20 \times 10}$$

Results:

The results of physico chemical analysis were showed in table (2)

CHEMICAL ANALYSIS

5.3 CHEMICAL ANALYSIS OF *PATTAI VALLATHAGI*

The chemical analysis of *PATTAI VALLATHAGI* was carried out in Bio-chemistry Lab, National Institute Of Siddha .

S.No	EXPERIMENT	OBSERVATION	INFERENCE
1.	Physical Appearance of extract	Light brown in colour	
2.	**Test for Slicate** a. A 500mg of the sample was shaken well with distilled water.	Sparingly soluble	Absence of Silicate
3.	**Action of Heat:** A 500mg of the sample was taken in a dry test tube and heated gently at first and then strong.	No White fumes evolved. No brown fumes evolved.	Absence of Carbonate Absence of Nitrate.
4.	**Flame Test:** A 500mg of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame.	No bluish green flame	Absense of copper
5.	**Ash Test:** A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited.	Appearance of yellow colour flame	Absense of sodium

Preparation of Extract:

5gm of Pattai Vallathagi was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and chemical constituents in it.

S.No	EXPERIMENT	OBSERVATION	INFERENCE
	I. Test For Acid Radicals		
1.	**Test For Sulphate:** a.2 ml of the above prepared extract was taken in a test tube to this added 2 ml of 4% dil ammonium oxalate solution b. 2 ml of the above prepared extract is added with 2 ml of diluted HCL is added until the effervescence ceases off. Then 2 m of Barium Chloride solution is added.	Cloudy appearance present A white precipitate insoluble in con.HCL was obtained.	**Presence of Sulphate** **Sulphate was confirmed**
2.	**Test For Chloride:** 2ml of the above prepared extracts was added with 2ml of dil-HCl is added until the effervescence ceases off..	No cloudy appearance	Absence of Chloride
3	**Test for phosphate :** 2ml of the extract were treated with 2 ml of dil.ammonium molybdate solution and 2 ml of con .HNo3	No cloudy yellow appearance formed	Absence of phosphate
4.	**Test For Carbonate:** 2ml of the extract was treated with 2ml dil. magnesium sulphate solution	No cloudy appearance.	Absence of carbonate
5.	**Test For Nitrate:** 1gm of the extract was heated with copper turning and concentrated H_2So_4 and viewed the test tube vertically down.	No Brown gas was evolved	Absense of nitrate
6.	**Test For Sulphide:** 1gm of the extract was treated with 2ml of con. HCL	No rotten egg smelling gas was evolved	Absence of sulphide
7.	**Test For Fluoride & Oxalate:** 2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.**	No cloudy appearance.	Absence of fluoride and oxalate
8.	**Test For Nitrite:** 3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution is placed.	No characteristic changes	Absence of nitrite
9.	**Test For Borate:** 50mg of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame.	bluish green colour flame not appeared	Absence of borate

II. Test For Basic Radicals			
1.	**Test For Lead:** 2ml of the extract was added with 2ml of dil.potassium iodine solution.	No Yellow precipitate was obtained	Absence of lead
2.	**Test For Copper:** **a.** One pinch (25mg) of extract was made into paste with con. HClin a watch glass and introduced into the non-luminuous part of the flame.	No blue colour precipitate	Absence of copper
3.	**Test For Aluminium:** To the 2ml of extract dil.sodium hydroxide was added in 5 drops to excess.	Shows characteristic changes	Absence of Aluminium.
4.	**Test For Iron:** a. To the 2ml of extract add 2ml of dil.ammonium solution b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added	Mild Red colour appeared	**Presence of Iron**
5.	**Test For Zinc:** To 2ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil .ammonium chloride is added.	NoWhite precipitate was formed	Absence of Zinc
6.	**Test For Calcium:** 2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution	Cloudy appearance and white precipitate was formed	**Presence of calcium**
7.	**Test For Magnesium:** To 2ml of extract dil.sodium hydroxide solution was added in drops to excess.	No White precipitate was obtained	Absence of magnesium
8.	**Test For Ammonium:** To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution are added.	No Light Brown colour appeared	Absence of ammonium
9.	**Test For Potassium:** A pinch (25mg) of extract was treated of with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil. glacial acetic acid.	Yellow precipitate was obtained	**Presence of potassium**
10.	**Test For Sodium**: 2 pinches (50mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner.	No yellow colour flame evolved.	Absence of sodium
11.	**Test For Mercury**: 2ml of the extract was treated with 2ml of dil. sodium hydroxide solution.	No Yellow precipitate was obtained	Absence of Mercury

12.	**Test For Arsenic:** 2ml of the extract was treated with 2ml of dil.sodium hydroxide solution.	No Brownish red precipitate was obtained	Absence of arsenic
III. Miscellaneous			
1.	**Test For Starch:** 2ml of extract was treated with weak dil.Iodine solution	Blue colour developed	**Presence of starch**
2.	**Test For Reducing Sugar:** 5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour changes are noted.	No Brick red colour was developed	Absence of reducing sugar
3.	**Test For The Alkaloids**: a) 2ml of the extract was treated with 2ml of dil.potassium lodide solution. b) 2ml of the extract was treated with 2ml of dil.picric acid. c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid.	No red colour developed	**Presence of Alkaloid**
4.	**Test For Tannic Acid:** 2ml of extract was treated with 2ml of dil.ferric chloride solution	Blue-black precipitate was obtained	**presence of Tannic acid**
5.	**Test For Unsaturated Compound:** To the 2ml of extract 2ml of dil.Potassium permanganate solution is added.	Potassium permanganate is not decolourised	Absence of unsaturated compound
6.	**Test For Amino Acid:** 2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent is added**.**	No violet colour	Absence of amino Acid
7.	**Test For Type Of Compound:** 2ml of the extract was treated with 2 ml of dil.ferric chloride solution.	No green and red colour No Violet colour developed No Blue colour developed.	Absence of quinolepinephrine pyrocatecho antipyrine Aliphatic amino acid and meconic acid. Apomorphine salicylate and Resorcinol are absent Morphine, Phenol cresol and hydrouinone are present.

Results:

The results of acid and basic radicals were showed in tables (3-5)

TLC/HPTLC FINGER PRINT ANALYSIS

5.4 TLC/HPTLC FINGER PRINT ANALYSIS

Thin layer chromatography (TLC) is a chromatographic technique used to separate the components of a mixture using a thin stationary phase supported by an inert backing. It may be performed on the analytical scale as a means of monitoring the progress of a reaction, or on the preparative scale to purify small amounts of a compound.

TLC/HPTLC is an analytical tool widely used because of its simplicity, relative low cost, high sensitivity, and speed of separation. TLC/HPTLC functions on the same principle as all chromatography: a compound will have different affinities for the mobile and stationary phases, and this affects the speed at which it migrates. The goal of TLC/HPTLC is to obtain well defined, well separated spots.

Retention Factor

After a separation was complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent. The Rf formula is

Rf = distance traveled by sample /distance traveled by solvent

The Rf value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions.

The compound with the larger Rf value is less polar because it does not stick to the stationary phase as long as the polar compound, which would have a lower Rf value

HEAVY METAL ANALYSIS

5.5 HEAVY METAL ANALYSIS

The procedure recommended for analysis of Heavy Metals like Lead and Cadmium in WHO, 1998 and AOAC, 2005. [20]

INSTRUMENT DETAILS:

Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectometer (AAS) was used for the analysis. The operating parameters:

Lead and Cadmium:

Instrument technique : Flame technique
Wavelengt (Lead) : 217 nm
Wavelengt (Cadmium) : 228.8 nm
Slit width : 0.5 mm
Lamp current (Pb) : 4.0 mA
Lamp current (Cd) : 3.0 mA
Carrier gas and flow rate : Air and Acetylene, 1.1 L/min

Mercury:

Instrument technique : Cold vapour technique
Wavelength : 253.7 nm
Slit width : 0. 5 mm
Lamp current : 3.0 mA,
Carrier gas and flow rate : Argon, 1.1 L/min
Flow rate : 5ml/min

Arsenic:

Instrument technique : Flame vapour technique
Wavelength : 193.7 nm
Slit width : 0. 5 mm
Lamp current : 6.0 mA,
Carrier gas and flow rate : Acetylene, Argon, 1.1 L/min
Flow rate : 5ml/min

The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

Result : The results of heavy metals were showed in table (7)

5.6 Microbial Analysis:

Microbial analysis was carried for determination of microbial contamination as per procedures of Indian pharmacopoeia 2010 and WHO Guideline . The test included total bacterial count, total fungal count, identification of specified organisms such as Escherichia coli, Salmonella sp., Staphylococcus aureus and Enterobacteriaceae,

Result :

The results of microbial analysis were showed in table (8)

ELEMENTAL ANALYSIS

5.7 ELEMENTAL ANALYSIS

The analysis of heavy metals and trace elements were estimated by using Inductively coupled plasma optical emission spectrometry (ICP- OES). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

INDUCTIVELY COUPLED PLASMA OPTICAL EMISSIOS SPECTROMETRY (ICP-OES)

Introduction

The element composition of a sample is often an important part of the information needed to assess its properties. Hence there is a need for scientific instrumentation like ICP-OES which plays a pivotal role in the determination of these elements. ICP-OES is widely employed for the estimation of metals and metallolids at trace, minor and major concentration.

Principle

In this technique, the high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons. The atoms of each element in the sample emit specific wavelength of light. The emission spectrum from the plasma is dispersed by an optical spectrometer , so that intension of the individual wavelength can be measured . the number of photos emitted is directly proportional to the concentration of the element. The photos may be detected either sequentially or simultaneously. Quantitative analysis is achieved by measuring the intensity of these specific wavelength and after performing the calibration using known standards.

Identifying the presence of emission at the wavelength characteristic of the element of interest obtaining quantitative information i.e, how much of an element is in sample can be accomplished using plots of emission intensity versus concentration called calibration curves.

Sample preparation – Microwave Digestion

- Weight 0.25 g of test sample and transfer into a liner provided with instrument.
- Slowly add 9ml of Nitric acid or sulphuric acid such that no piece of sample sticks on the slide.

- Mix thoroughly and allow reacting for few minutes.
- Place the liner in the vessel jacket.
- Close the screw cap hand- tight in clockwise direction.
- Seal the vessel and placed in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes, hold at 180°C for least 10 minutes. Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made upto 100ml with Millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly.

Perkin Elmer Optima 5300DV was used for standard ICP-OES analysis. The optimized operating conditions are given in table 1, and the test *Pattai Vallathagi* underwent microwave digestion for sample preparation.

Result :

The results of elemental analysis were showed in table (9)

PARTICLE SIZE ANALYSIS

5.8 ANALYSIS OF PARTICAL SIZE

SCANNED ELECTRON MICROSCOPY (SEM)

The partical size of the *Pattai Vallathagi* was determined using High resolution scanning electron microscopy (HR SEM). The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.

Experimental procedure :

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways :-

- Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.
- Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection.The X-ray emitted are characteristic of the elements in the top few μm of the sample.

The SEM is carried out by using FEI Quanta FEG 200-High Resolution Instrument.

Resolution : 1.2 nm gold particle separation on a carbon substrate

Magnification : From a min of 12 X to greater than 1,00,000 X.

Method :

A representative portion of each sample was sprinkled on to a double side carbon tape and mounted on aluminium stubs, in order to get a higher quality secondary electron image for SEM examination.

Sample preparation :

Sample preparation can be minimal or elaborate for SEM analysis, depending on the nature of the samples and the data required. Minimal preparation includes acquisition of

a sample that will fit into the SEM chamber and some accommodation to prevent charge build-up on electrically insulating samples.

Calculation of the particle size:

The horizontal line in the right corner of the micrograph corresponds to micron in length would be given. A comparison could be made between the length of the particles visible in the micrograph with this line and the length of the particle was calculated

Result :

The results of particle size were showed in fig (1)

TOXICOLOGICAL STUDIES

6. TOXICOLOGICAL EVALUATION OF *PATTAI VALLATHAGI* IN RODENTS

Introduction:

Safety is a fundamental principle in the provision of traditional medicines and herbal products for health care and a critical component of quality control. OECD guidelines provide practical and technical guidance for monitoring the safety of traditional medicines within pharmacovigilance systems. The safety monitoring of traditional medicines is compared and contrasted with that of other medicines, currently undertaken in the context of the WHO International Drug perspective.

Scope of work:

Monitoring Programme, while there are regulatory and cultural differences in the preparation and use of different types of medicines, they are all equally important from a pharmacovigilance

Assurance of safety, quality and efficacy of Indian System of Medicines (ISM) is the key issue that needs to be addressed while conducting toxicity studies. It is an essential step, which will strengthen the acceptance of Siddha medicines by scientific community. Information of toxicity and adverse effects of these formulations are lacking. Some of the formulations are proved to be effective in various animal studies and many more are yet to be tested.

Hence, the present study was carried out to evaluate the Preclinical animal toxicity studies of ***PATTAI VALLATHAGI*** in rodents.

Plan of work:

The following studies were carried out on *PATTAI VALLATHAGI*

- Acute oral toxicity – OECD 423
- Repeated dose 28 day oral toxicity study – OECD 407
- Reapted dose 90 days oral toxicity study-OECD 408

ACUTE TOXICITY STUDY

6.1 ACUTE ORAL TOXICITY STUDY OF *PATTAI VALLATHAGI*

(OECD GUIDELINE - 423)

Aim :

To evaluate the acute and sub acute sub chronic toxicities of siddha drug "*Pattai Vallathagi"* in wistar albino rats. The drug *Pattai Vallathagi* was prepared by the method prescribed in standard text books of siddha medicine.

experiment procedure:

Acute toxicity study was carried out according to the OECD (Organization of Economic Co-operation and Development) guidelines 423. Healthy female rats, weighing 150–200 g, were selected and oral administration of the single doses of *Pattai Vallathagi* were done aseptically by water.

Experimental animals:

Albino rats (wistar rats) of either sex weighing (150-200 g) were procured from animal housing facility, K.K college of pharmacy, Gerugambakkam, Chennai. All animals were placed in a polypropylene cages in a controlled room temperature 24° C±1° C and relative humidity of 60-70 % in animal house. The animals were maintained in standard pellet diet and water ad libitum .They were acclimatized to laboratory condition for seven days before commencement of the experiment.

All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and Supervision of Experiments on Animals **(KKCP/2015/026-).**Animal experimentation protocols are approved by Institutional Animal Ethical Committee.

Administration of doses:

Pattai Vallathagi was administered as a single oral dose by gavage using a feeding needle. Animals were fasted prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. After the substance has been administered, food was withheld for further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously observed as per the guideline after substance administration.

An oral (p.o) dose of 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg was administered step by step according to the guidelines. The general behaviors of the rats were continuously monitored for 1 h after dosing, periodically during the first 24 h (with special attention given during the first 4 hours and then daily thereafter, for a total of 14 days. Changes in the normal psychomotor activity and external morphology and their body weights were monitored periodically before dosing and the time at which signs of toxicity or mortality were recorded.

The visual observations included skin changes, morbidity, aggressiveness, sensitivity to sound and pain, as well as respiratory movements were recorded. They were deprived of food, but not water 12 h prior to the administration of the test substance. Finally, the number of survivors was noted after 24 h and these animals were then maintained for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Test Substance	:	*Pattai Vallathagi*
Animal Source	:	Animal house of King Institute of Preventive Medicine
Animals	:	Male and Female Wistar Albino Rats
Age	:	More than 8 weeks
Acclimatization	:	Seven days prior to dosing.
Veterinary examination	:	Prior to and at the end of the acclimatization period.
Identification of animals	:	By cage number, animal number and individual marking on fur.
Diet	:	Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore
Water	:	Potable water in polypropylene bottles *ad libitum.*
Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	Between 20 & 24°C,
Relative humidity	:	Between 30% and 70%,
Dark and light cycle	:	Each of 12 hours.

Number of animals and dose levels:

Three animals were used for each step. The dose level used as the starting dose was selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level was most likely to produce mortality in some of the dosed animals. The available information suggests that mortality is likely at the highest starting dose level 2000mg/kg body weight, so the trial or limit test was conducted. The time interval between treatment groups is determined by the onset, duration and severity of toxic signs.

OBSERVATIONS:

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total period of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed.

All observations were systematically recorded with individual records being maintained for each animal. Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somato motor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death should was recorded. From the maximum dose 1\5th or 1\10th of the dose was considered as therapeutic dose for further study

Results :

All date were summarized in table (10)

REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY

6.2 REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF PATTAI VALLATHAGI (OECD GUIDELINE - 408)

experiment procedure:

Sub-acute toxicity study was carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *Pattai Vallathagi* was administered to rats at the dose of 900mg /kg and 1800mg/kg continuously for 28 days. The animals were observed daily for gross behavioural changes and other sign of sub acute toxicity. The weight of the each rat was recorded on day 0 and weekly throughout the course of the study, Food and water consumption per rat was calculated. At the end of 28 days they were fasted overnight ,each animal were anaesthetized with diethyl ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Test Substance	:	*Pattai Vallathagi*
Animal Source	:	Animal house of King Institute of Preventive Medicine
Animals	:	Male and Female Wistar Albino Rats
Age	:	More than 8 weeks
Acclimatization	:	Seven days prior to dosing.
Veterinary examination	:	Prior to and at the end of the acclimatization period.
Identification of animals	:	By cage number, animal number and individual marking on fur.
Diet	:	Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore
Water	:	Potable water in polypropylene bottles *ad libitum.*
Housing & Environment	:	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	:	Between 20 & 24°C,
Relative humidity	:	Between 30% and 70%,
Dark and light cycle	:	Each of 12 hours.
Duration of study		28 days

Justification for Dose Selection:

The results of acute toxicity study in rats indicated that *Pattai Vallathagi* was non toxic and no behavioral changes was observed up to the dose level of 2000mg/kg body weight. The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and administration of dose:

Pattai Vallathagi at two doses level 900mg/kg and 1800mg/kg respectively were prepared.. The test substance were freshly prepared every day for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals:

Ten Rats (Five Male and Five Female) in each group randomly divided into three groups for dosing up to 28 days. Animal's acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant.

OBSERVATIONS:

Experimental animals were kept under observation throughout the course of study for the following:

i)Body Weight:

Weight of each rat was recorded on day 0 at weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percent body weight gain were calculated table (11)table (19)

ii) Food and water Consumption:

The quantity of food consumed by groups consisting of ten animals of for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups tables (12,13)

iii)Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

iv)Mortality:

All animals were observed twice daily for mortality during entire course of study.

v)Laboratory investigation:

Following laboratory investigations were carried out on day 29 in animals fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then anesthetized with ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of haematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

Haematological Investigations:

Blood samples of control and experimental rats were analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count, Mean corpuscular volume (MCV) and packed cell volume (PCV). From the estimated values of RBC count (millions/mm3) and PCV (volumes percent), mean corpuscular volume (MCV) was calculated.

Biochemical Investigations:

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, uric acid, creatinine, triglyceride, cholesterol and glucose levels by using standard methods. Activities of glutamate oxaloacetate transaminase/ Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed on day 29. Necropsy of all animals were carried out and the weights of the organs including liver, kidneys, adrenals, spleen, brain, heart, uterus and testes/ovaries were recorded. The relative organ weight of each animal was then calculated as follows;

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100$$

Histopathsology:

Histopathological investigation of the vital organs were done. The organ pieces(3-5µm thick) of the highest dose level of 400mg /kg were preserved and were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technic on and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included heart, kidneys, liver ,Spleen, Brain of the animals were preserved they were subjected to histopathological examination.

Statistical analysis:

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way Anova Followed by Dunnet't' test using a computer software programme. (Graph Pad Prism 5.0)

Result :

All data were summarized in tabular form (Table 14-18)

REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY

6.3 REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY OF *PATTAI VALLATHAGI* (OECD GUIDELINE - 408)

Test Substance	:	***PATTAI VALLATHAGI***
Animal Source	:	Animal house of King Institute of Preventive Medicine
Animals	:	Wister Albino Rats (Male -3, and Female-3)
Age	**:**	6-8 weeks
Body Weight	**:**	150-200gm.
Acclimatization	**:**	Seven days prior to dosing.
Veterinary examination	**:**	Prior and at the end of the acclimatization period.
Identification of animals	**:**	By cage number, animal number and individual marking by using Picric acid.
Diet	**:**	Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:**	Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:**	The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:**	between 22ºC $\pm$ 3ºC.
Relative humidity	**:**	between 30% and 70%,
Air changes	**:**	10 to 15 per hour
Dark and light cycle	**:**	12**:**12 hours.
Duration of the study	**:**	**90 Days.**

METHODOLOGY

Randomization, Numbering and Grouping of Animals:

24 Wistar Albino Rats (12M + 12F) were selected and divided into 4 groups. Each group consist of 6 animals (Male -3, and Female-3) (IAEC Approval no NIS/IAEC-I/2016/01).Ist group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study.They were low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose and the body surface area of the rat (0.018). i.e X dose is 180 mg/kg, 5X dose is900 mg/kg,10X dose is 1800mg/kg.

Preparation and Administration of Dose:

Pattai vallathagi was suspended with distilled water to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

OBSERVATIONS:

Experimental animals were kept under observation throughout the course of study for the following:

- **Body Weight:**

  Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study. (Table -20)

- **Clinical signs**:

  All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

- **Mortality:**

  All animals were observed twice daily for mortality during entire course of study.

- **Laboratory Investigations:**

  Following laboratory investigations were carried out on day 91 in animals'fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

- **Haematological Investigations:**

  Haematological parameters were determined using Haematology analyzer.

- **Biochemical Investigations:**

  Biochemical parameters were determined using auto-analyzer.

- **Histopathology:**

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technic on and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

- **Statistical analysis:**

Findings such as clinical signs of intoxication, body weight changes, haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet't'test using a computer software programme -INSTAT-V3 version.

Result :

All data were summarized in tabular form (Table 21-25)

ANTI-INFLAMMATORY ACTIVITY

7.1 ANTI-INFLAMMMATORY ACTIVITY OF PATTAI VALLATHAGI IN WISTER ALBINO RATS

AIM:

To evaluate the anti - inflammatory activity of Pattai Vallathagi in Wistar albino rats by ***Cotton pellet granuloma method***.

Selection of Experimental animals:

The experimental protocol was submitted and approved by institutional Ethical Committee, (IAEC approval No KKCP/2015/026). Wistar albino rats (150- 200 gm) of approximate same age were employed in this investigation. The animals were obtained from animal house, K.K college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2˚C and relative humidity of 30-70% at 12 :12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet.

Experimental Design for Cotton pellet granuloma model

The animals were divided into four groups each group consists of 6 animals.

Group-I : Control - control received distilled water (dose: 10 ml/kg).
Group-II : Standard drug - Animals treated with Dexamethasone (dose: 0.5 mg/kg).
Group-III : Animals treated with Pattai Vallathagi (200 mg/kg).
Group-IV : Animals treated with Pattai Vallathagi (400 mg/kg)

Experimental procedure

Inflammation was induced by cotton pellet granuloma model. This method was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia by using blunted forceps, subcutaneous tunnel was made and sterilized cotton pellets (10 ± 1 mg) were implanted in the axilla and groin region of the rat. After recovering from anaesthesia, animals were treated orally with vehicle control (Distilled water 10 ml / kg), Dexamethasone 0.5 mg/kg, low dose (200mg/kg) and high dose (400mg/kg) of *Pattai Vallathagi* for consecutive 7 days, once per day. They were sacrificed on day 8th by cervical dislocation and the pellets were removed and immediately the wet weight was taken, freed from extraneous tissue and dried at 600C for 24 hrs. The percentage inhibition of wet weight and dry weight of the granuloma were calculated and compared.

$$\text{Percentage inhibition (\%)} = \frac{\text{Control - Treated}}{\text{Control}} \times 100$$

Statistical analysis

Results were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied.

Result :

Results of anti inflammatory activity by cotton pellet granuloma method showed in table (26)

ANALGESIC ACTIVITY

7.2 ANALGESIC ACTIVITY OF *PATTAI VALLATHAGI* IN SWISS ALBINO MICE

AIM:

To evaluate the Analgesic activity of *Pattai Vallathagi* in Swiss albino mice by ***Eddy's Hot plate method***.

Selection of Experimental animals:

Healthy Swiss albino mice of either sex weighing (20-25gms) were used for this study. The animals were obtained from animal house, K.K college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2c and relative humidity of 30-70%. At 12 :12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) InstitutionalAnimal Ethics Committee KKCP/20/026 of K.K college of Pharmacy and were in accordance with the guidelines of the IAEC.

Evaluation of Analgesic activity

Pain is the part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contralateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain

Eddy's Hot plate method:

Principle:

Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal – using radiant heat as a source of pain, chemical – using irritants such as acetic acid and bradykinin and physical pressure – using tail compression.

The hot plate test is a test of the pain response in animals. It is used in basic pain research and in testing the effectiveness of analgesics by observing the reaction to pain caused by heat.

They used a behavioral model of nociception where behaviors such as jumping and hind paw-licking are elicited following a noxious thermal stimulus. Licking is a rapid response to painful thermal stimuli that is a direct indicator of nociceptive threshold. Jumping represents a more elaborated response, with a latency and encompasses an emotional component of escaping.

Animals

Mice 20-25 g were grouped in four groups, six animals in each group.

Grouping:

Group I : **Control** - distilled water (10ml/kg, p.o.),

Group II : **Standard drug -** Pentazocine (5mg/kg, p.o.)

Group III : **Received** *Pattai Vallathagi* (200mg/kg)

Group IV : **Received** *Pattai Vallathagi* (400mg/kg)

Equipment:

Eddy's Hot plate

Procedure:

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55±f C. Jumping response was seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time. The time obtained was considered the basal / normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30 , 60 ,90 ,120 and 150 mins interval after drug administration.

Statistical analysis

Results were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied.

Result :

The results of analgesic activity by Eddy's Hot plate method was represented in table (27)

ANTI- HISTAMINE ACTIVITY

7.3 ANTI-HISTAMINE ACTIVITY OF *PATTAI VALLATHAGI* IN GUINEA PIG

Experimental animals

Guinea pig weighing 400-450g were procured from the animal house in K.K college of pharmacy . They were fed with food and water *ad libitium*. The animals were acclimatized for atleast one week in lab condition before commencement of the experiment in standardlaboratory conditions 12 ± 01 hour day and night cycle, maintained at 25 ± 30C and 40 to 60% humidity. The animal protocol was approved by the Institutional Animal Ethics Committeewith approval number; IAEC-KKCP/2015/026.

Experimental methods(Isolated guinea pig ileum preparation)

Procedure:

Preparation of test drug and Histamine

Test drug was dissolved in distilled water and desired concentration was prepared. Histamine was dissolved in physiological saline. Physiological saline was widely recommended as it is known to be compatible with human tissue, and isotonicity with body fluids.

Anti histaminic activity: (Effect of test drug on the guinea pig ileum preparation:-

To assess the antihistaminic activity of the test drug, the experiment was carried out on isolated guinea pig ileum Overnight fasted guinea pig was stunned by head blow, neck vessels cut and the animal is cut open. Abdomen was opened through a midline incision, the ileocaecal junction exposed; the terminal ileum was cut after discarding 10cm nearest to the ileocaecal junction. Isolatedileum was placed on a petri dish containing Tyrode solution(Nacl 8.0, Kcl- 0.2, Cacl2-0.2, Mgcl2 -0.1, NaHCO3 -1.0, NaH2PO4 -0.05 and Glucose-1.0gmper liter) at37°C. A 2.5 cm long piece of the distal part of the ileum was used for the study. Experiments were performed in organ bath containing Tyrode solution at 37°C and bubbled with Oxygen(air,O2, or 5% CO2 in O2 used for mammalian smooth muscles). The responses were recorded on kymograph paper with frontal writing lever having a 4-7 magnification and 0.5gm initial tension. The preparation was allowed to equilibrate for 30 -45 min ,increasing concentration of histamine was recorded with a contact time of 90 seconds. .The test drug *Pattai Vallathagi* were added to the reservoir and same doses of histamine were repeated in

the presence of *pattaivallathagi*. Then the standard drug chlorphenaraminemaleate(10µg/ml) was added and the same procedure was repeated .Responses to histamine were recorded as changes in height from baseline and expressed as percent of maximum response of the histamine. The CRC was constructed with a 20min rest between each. The mean maximal response obtained from the first concentration response curve was taken as the 100% response.

Statistical analysis:

The data was analyzed by one way ANOVA followed by dunnet test.

Results :

The results of Anti- histamine activity by ileum cut terminal method was represented in table (28)

RESULTS

8. RESULT

5.1 ORGANOLEPTIC CHARACTER*

Table:1. Organoleptic characters of *Pattai Vallathagi*

Colour	**Dark Brown**
Odour	**Pleasant**
Taste	**Characteristic taste**
Texture	**Semi solid**

5.2 PHYSICOCHEMICAL ANALYSIS*

Table :2 Physicochemical properties of *Pattai Vallathagi*

S.NO	Parameters	Results
1.	pH at 10% of aqueous solution	6.50
2.	Total Ash	2.40%
3.	Acid insoluble ash	0.19%
4.	Loss on drying@105°C	3.25%
5.	Specific gravity	1.10
6.	Fat content	39.47
7.	Reducing Sugar	3.00
8.	Total Sugar	15.51

****The Experimental Procedure was analyzed at Captain Srinivasa murti Reseach Institute of Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-106.**

5.3 Chemical Analysis of *Pattai Vallathagi*[*]

Table 3 : Results of Acid radicals studies

S.NO	Parameter	Observation	Result
1	Test for Sulphate	Cloudy appearance was formed	**Positive**
2	Test for Chloride	-	Negative
3	Test For Phosphate	-	Negative
4	Test For Carbonate	-	Negative
5	Test For Nitrate	-	Negative
6	Test for Sulphide	-	Negative
7	Test For Fluoride &oxalate	-	Negative
8	Test For Nitrite	-	Negative
9	Test For Borax	-	Negative

Interpretation

The acidic radicals test shows the presence of chemical **sulphate.**

Table 4 :Results of basic radicals studies[*]

S.NO	Parameter	Observation	Result
1	Test for Lead	-	Negative
2	Test for Copper	-	Negative
3	Test For Aluminium	-	Negative
4	Test For Iron.	Mild red colour appeared	**Positive**
5	Test For Zinc	-	Negative
6	Test for Calcium	Cloudy appearance and white precipitate was formed	**Positive**
7	Test For Magnesium	-	Negative
8	Test For Ammonium	-	Negative
9	Test For Potassium	Yellow precipitate was appeared	**Positive**
10	Test For Sodium	-	Negative
11	Test For Mercury	-	Negative
12	Test For Arsenic	-	Negative

Interpretation : The basic radical test shows the presence of **Iron, cacium, , Potassium** and absence of heavy metals such as lead, arsenic and mercury.

Table :5 Result for other constituents*

S. No	Parameter	Observation	Result
1.	Test for starch	-	-
2.	Test for reducing sugar	-	-
3.	Test for Alkaloids	No red colour developed	**Positive**
4.	Test for Amino acid	-	-
5.	Test for Tannic acid	Blue–black precipitate was obtained	**positive**
6.	Test for type of compounds	-	-

Table shows the presence of **alkaloids** and **tannic acid** in *Pattai Valathagi*

***The chemical analysis of was carried out in Bio-chemistry Lab, National Institute Of Siddha .**

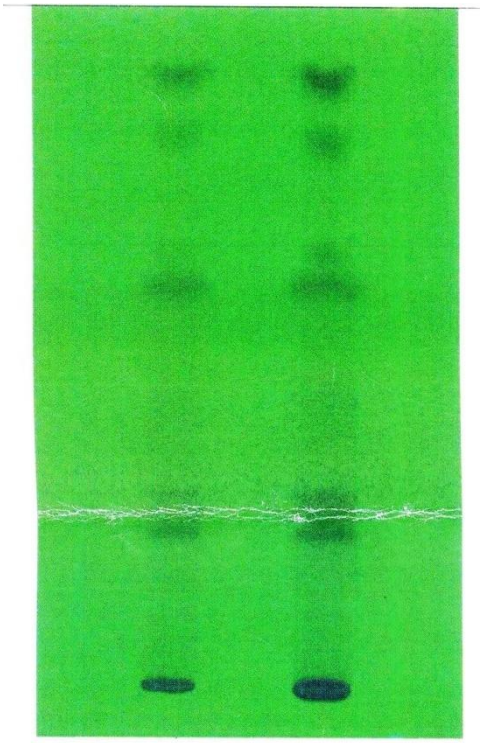
5.4 TLC and HPTLC analysis

The procedure recommended for the analysis of TLC and HPTLC analysis as per Wagner H and Bladt S, 1996

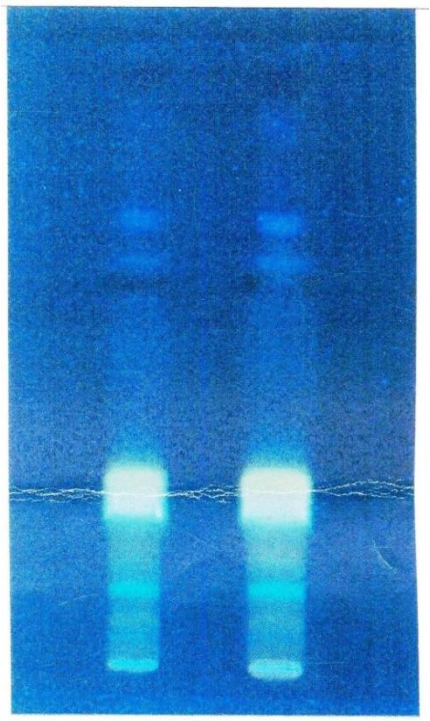
Table:6

UV-254nm	UV-366nm	V-S Reagent
0.25 Green	0.88 Blue	0.12 Violet
0.31 Green	0.14 Blue	0.24 Violet
0.46 Green	0.18 Blue	0.31 Pink
0.65 Green	0.26 Yellow	0.51 Blue block
0.69 Green	0.31 Yellow	0.63 Brown
0.86 Green	0.61 Blue block	0.67 Blue block
0.95 Green	0.69 Blue	0.74 Pink
	0.74 Blue	0.85 Violet

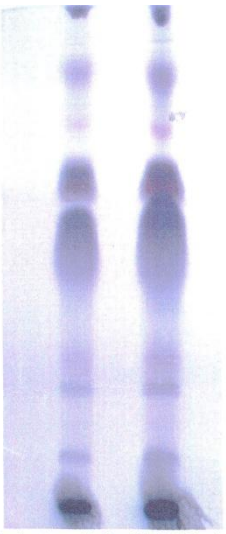
TLC PHOTODOCUMENTATION OF DTL SAMPLE CODES 1510352



UV 254 nm



UV 366 nm

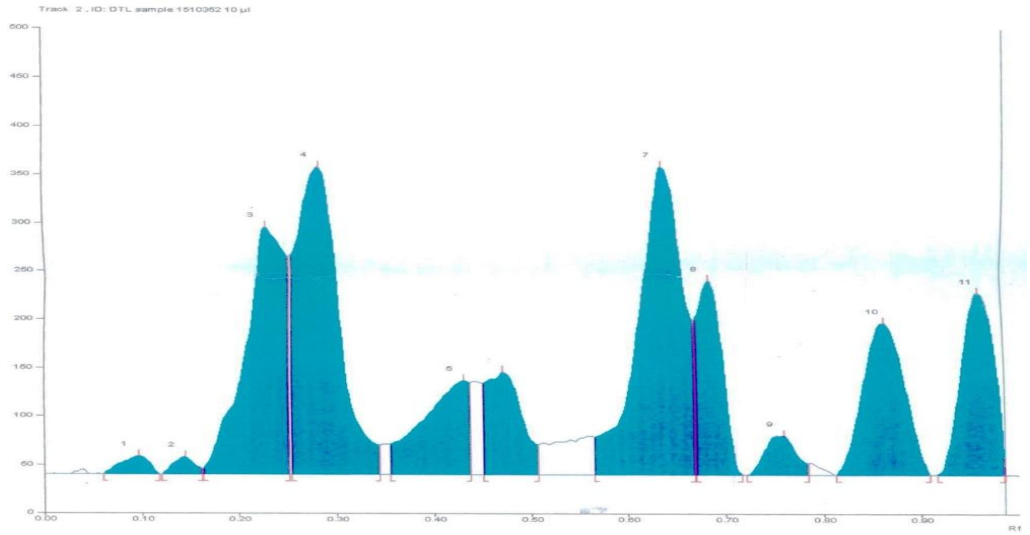


Derivatization with Vanillin-Sulphuric acid

Solvent system : *Toluene : Ethyl acetate (9:1)*

Track 1: 5 µ1 Sample**, Track 2:** 10 µ1 Sample

HPTLC finger print profile of DTL Sample Coded 1510352- at- 254 nm



Track 2, ID: DTL sample 1510352 10 µl

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %		Assigned substance
1	0.06 Rf	0.3 AU	0.10 Rf	18.8 AU	1.10 %	0.12 Rf	0.3 AU	515.5 AU	0.81 %		unknown *
2	0.12 Rf	0.1 AU	0.14 Rf	18.4 AU	1.07 %	0.16 Rf	6.2 AU	366.2 AU	0.58 %		unknown *
3	0.16 Rf	6.3 AU	0.23 Rf	255.6 AU	14.86 %	0.25 Rf	25.2 AU	9879.4 AU	15.55 %		unknown *
4	0.25 Rf	225.6 AU	0.28 Rf	318.3 AU	18.51 %	0.34 Rf	30.5 AU	13180.5 AU	20.75 %		unknown *
5	0.35 Rf	31.3 AU	0.43 Rf	97.0 AU	5.64 %	0.44 Rf	35.2 AU	4365.6 AU	6.87 %		unknown *
6	0.45 Rf	94.7 AU	0.47 Rf	106.3 AU	6.18 %	0.51 Rf	31.0 AU	3702.6 AU	5.83 %		unknown *
7	0.56 Rf	39.5 AU	0.64 Rf	319.4 AU	18.57 %	0.67 Rf	59.9 AU	13679.8 AU	21.53 %		unknown *
8	0.67 Rf	160.8 AU	0.69 Rf	200.5 AU	11.66 %	0.72 Rf	1.4 AU	4666.0 AU	7.34 %		unknown *
9	0.72 Rf	0.1 AU	0.76 Rf	40.7 AU	2.37 %	0.78 Rf	12.4 AU	1201.7 AU	1.89 %		unknown *
10	0.81 Rf	0.4 AU	0.86 Rf	156.9 AU	9.12 %	0.91 Rf	0.9 AU	6154.3 AU	9.69 %		unknown *
11	0.92 Rf	1.0 AU	0.96 Rf	188.0 AU	10.93 %	0.98 Rf	18.6 AU	5819.5 AU	9.16 %		unknown *

***The Experimental Procedure was analyzed at Captain Srinivasa Murthi Research Institute of Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-106.**

5.5 Heavy Metals Analysis of *Pattai vallathagi*[*]

Table 7 :

S. No.	Name of the Element	Results	Permissible Limit
1	Lead	4.62ppm	10 ppm (WHO)
2	Cadmium	0.31ppm	0.3 ppm (WHO)
3	Mercury	0.012ppm	1 ppm (API)

From Table (7) the heavy metals in *Pattai Vallathagi* were found to be with in normal limits except Cadmium which is nearer to the permissible limit.

5.6 Microbial analysis of *Pattai Vallathagi* [*]

Table 8: Screening for Micro – organisms:

S.No	Parameters	Results	Permissible Limit for Internal use
1	Total Bacterial Count (TBC)	$0.5x\ 10^3$ CFU/g	10^5 CFU/g
2	Total Fungal Count (TBC)	$<10^3$	10^3 CFU/g
3	Enterobacteriaceae	Absent	10^3 CFU/g
4	*Escherichia coli*	Absent	10 CFU/g
5	*Salmonella spp*	Absent	Absent
6	*Staphylococcus aureus*	Absent	Absent

***The Experimental Procedure was analyzed at Captain Srinivasa murthi Research Institute of Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-106.**

5.7 ELEMENTAL ANALYSIS

The analytical results of heavy metals and trace elements in *Pattai Vallathagi* using ICP-OES are showed in table

Table 9. ICP- OES study results of *Pattai vallathagi*

S.No	Elements	Wavelength in nm	mg/L
1	Arsenic	As188.979	BDL
2	Calcium	Ca 315.807	32.860 mg/L
3	Cadmium	Cd 228.802	BDL
4	Copper	Cu 327.393	BDL
5	Lead	Pb 220.353	BDL
6	Mercury	Hg 253.652	BDL
7	Nickel	Ni 231.604	BDL
8	Potassium	K 766.491	04.621 mg/L
9	Phosphorus	P 213.617	16.941 mg/L
10	Sodium	Na 589.592	04.810mg/L
11	Sulphur	S180.731	81.374mg/L

BDL-Below Detection Limit, ppm –Parts per million

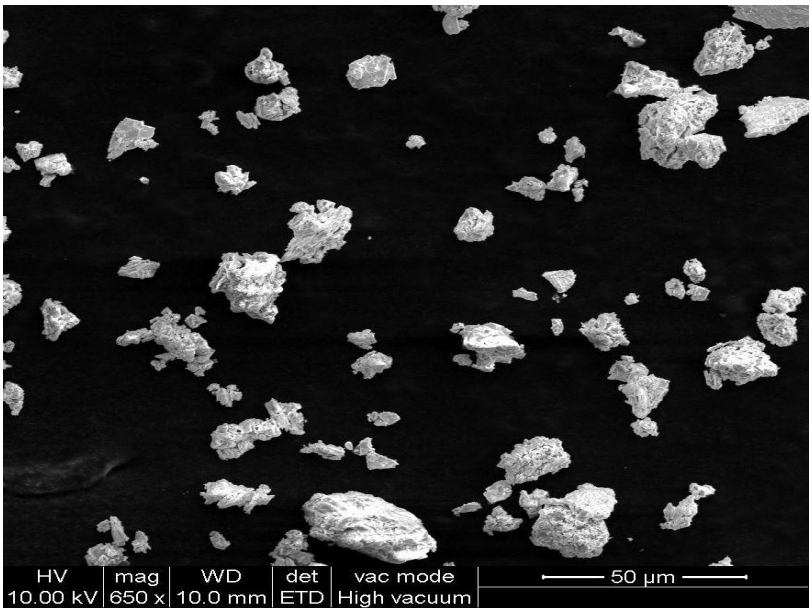
Table (9) : shows the quantitative analysis of the elements present in *Pattai Vallathagi*, The heavy metals were found to be with in normal limits.

***The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.**

SCANNED ELECTRON MICROSCOPY

5.8.Determination of particle size of *Pattai Vallathagi*

Figure 1



The picture shows that the particles are stabilize, have irregular morphology and distributed in near micron. ***Pattai Vallathagi*** has particle size of 1.2-2.4 µ

*****The Experimental Procedure was done at SAIF, IIT Madras, Chennai-36.**

ACUTE ORAL TOXICITY STUDY OF PATTAI VALLATHAGI*

Table 10 : Dose finding experiment and its behavioural Signs of Toxicity

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	5	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	50	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	300	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	2000	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1.Alertness 2.Aggressiveness 3.Pile erection 4.Grooming 5.Gripping 6.Touch Response 7.Decreased Motor activity 8.Tremors 9.Convulsions 10.Muscle Spasm 11.catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18. Writhing 19.Respiration 20.Mortality

\+ Presence Of Activity

_ Absence Of Activity

Result :

All data were summarized in the form of table (10)revealed no abnormal signs and behavioral changes in rats at the dose of 5, 50, 300 & 2000 mg/kg body weight administered orally.

***The Acute oral toxicity study was done at K.K College of Pharmacy, Gerugambakkam**

28 DAYS REPEATED ORAL TOXICITY STUDY OF PATTAI VALLATHAGI

Table 11 :Body weight (g) of wistar albino rats group exposed to Pattai Vallathagi for 28 days

DOSE	0	7	14	21	28
CONTROL	163.42±5.00	165.52±6.00	166.24±5.25	168.05±5.21	171.10±5.26
MID DOSE	165.17±1.15	166.41±5.11	170.12±5.72	172.61±6.00	174.51±5.28
HIGH DOSE	166.20±5.47	168.10±6.00	171.18±6.12	173.12±5.10	174.30±6.14

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

Table 12: Water intake (ml/day) of wistar albino rats group exposed to Pattai Vallathagi for 28 days

DOSE	DAYS				
	0	7	14	21	28
CONTROL	30.31±0.36	30.45±0.32	30.68±0.25	30.80±0.20	30.6±0.20
MID DOSE	31.02±0.48	31.19±0.32	31.16±0.16	31.36±0.08	31.01±0.16
HIGH DOSE	31.46±0.08	31.32±0.16	31.61±0.39	31.12±0.40	31±0.02

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

Table 13: Food intake (gms/day) of wistar albino rats group exposed to Pattai Vallathagi for 28 days

DOSE	DAYS				
	0	7	14	21	28
CONTROL	20.66±0.81	22.16±0.25	24.23±0.36	23.73±0.43	24.33±0.81
MID DOSE	21.12±0.23	22.71±0.16	24.81.±0.39	24.13±0.63	25.12±0.51
HIGH DOSE	21.67±0.15	22.62±0.13	25±0.15	24.65±0.57	25.92±0.44

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* p<0.05 ;**p<0.01.N=10

Table 14. Haematological parameters (28 days – sub acute)

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE
Total RBC count ($\times 10^6$ mm−3)	9.09±0.25	9.10±0.04	9.09±0.07
Total WBC Count ($\times 10^3$ mm−3).	11.41±0.21	11.52±0.11	11.66±0.43
Haemoglobin (Hb) (g/dl)	15.26±0.21	14.7±0.52	15.1±0.43
Platelets (×103 mm−3).	410.0±9.70	423.16±4.83	430.1±4.64
PCV %	39.11±0.34	39.25±0.41	39.86±0.18
MCV (ft)	51.02±0.21	51.43±0.29	51.8±0.21

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

Chart 1 : The mean value of Hb, T.RBC Count of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

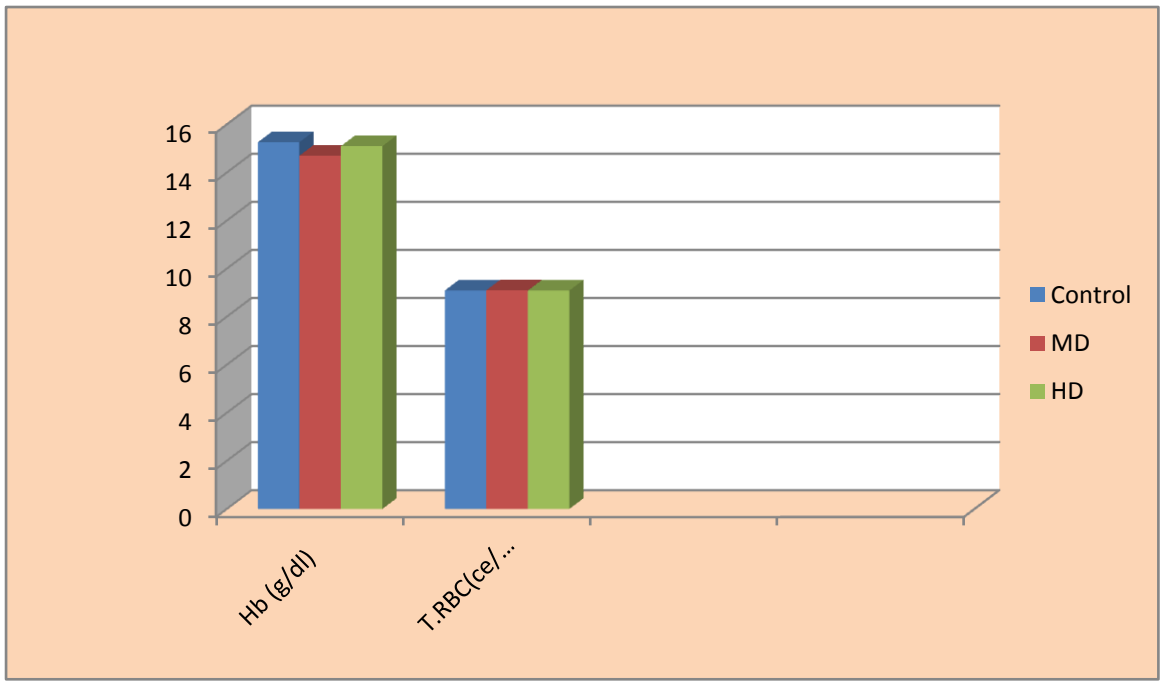


Chart 2 :The mean value of Platelets of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

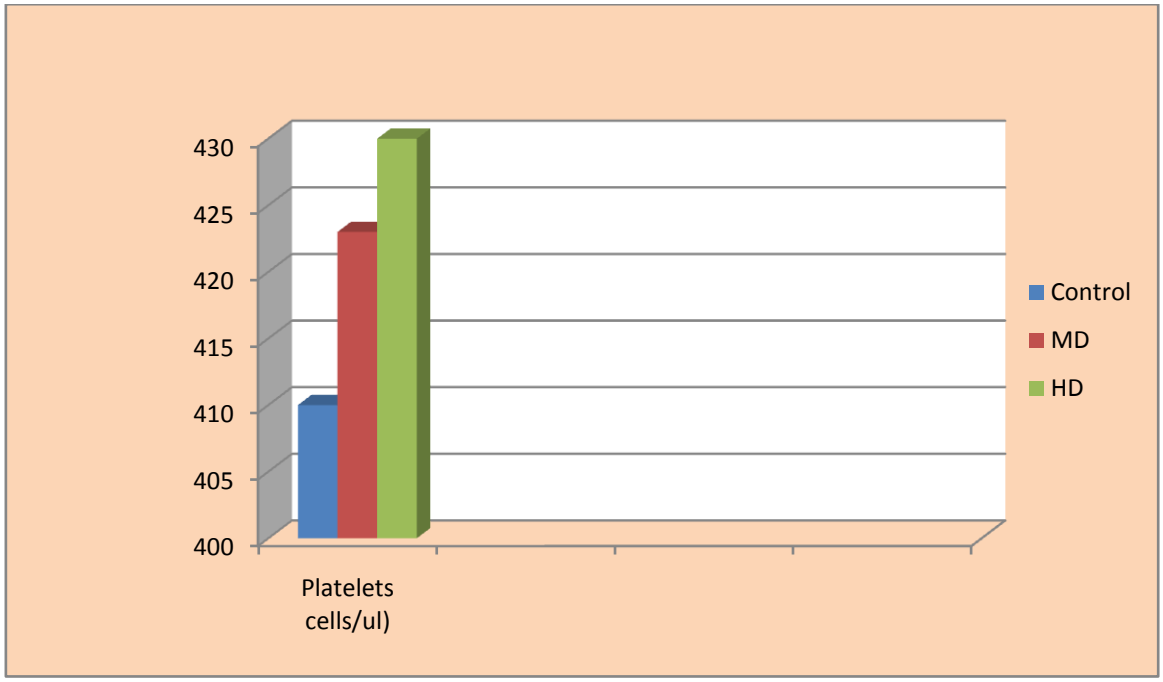


Chart 3 : The mean value of T.WBC Count,PCV, MCV of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

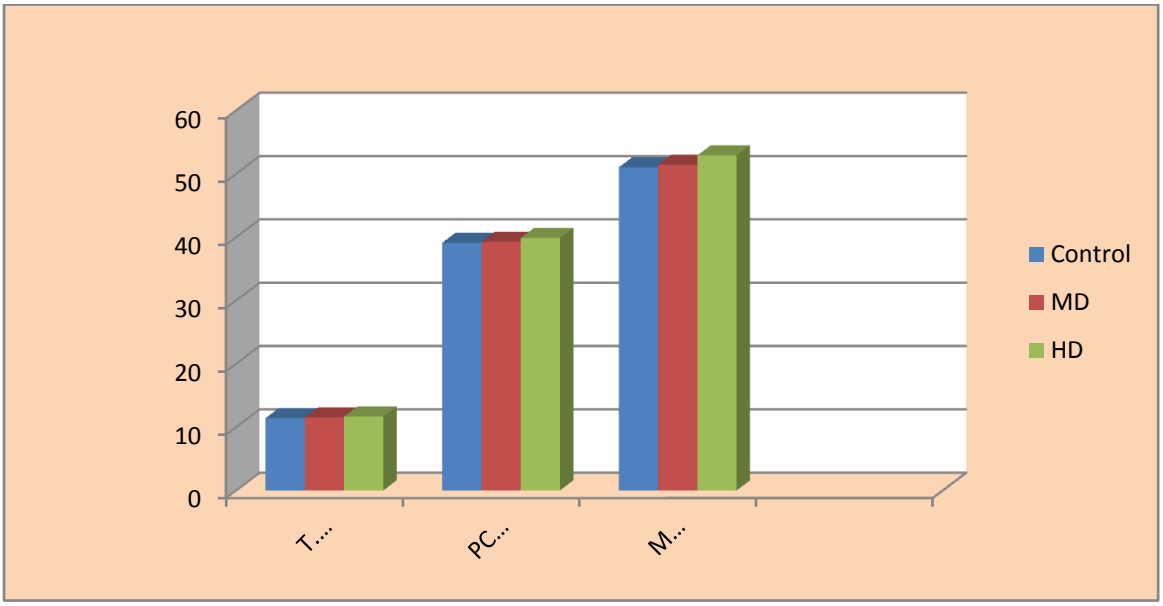


Table 15:Effect of treatment with Pattai Vallathagi on biochemical parameters:

Liver function test

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE
Total Bilirubin(mg/dl)	0.7±0.08	0.65±0.17	0.7±0.14
Bilirubin direct(mg/dl)	0.3±0.06	0.35±0.10	0.35±0.05
Bilirubin indirect(mg/dl)	0.4±0.06	0.31±0.10	0.35±0.10
ALP(U/L)	110.83±2.23	114.83±0.75	115.66+1.03
AST(U/L)	55.33±2.25	56.33±1.21	56.52±1.87
ALT(U/L)	68.38±0.57	68.21±0.95	68.66±1.03
Totalprotein(g/dl)	5.10±0.14	5.34±0.03	5.25±0.03
Albumin(g/dl)	3.19±0.02	3.20±0.01	3.23±0.02
Globulin(g/dl)	6.36±0.03	6.41±0.01	6.43±0.04

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

Chart 4 : The mean value of T.Bilirubin, Direct & Indirect Bilirubin of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

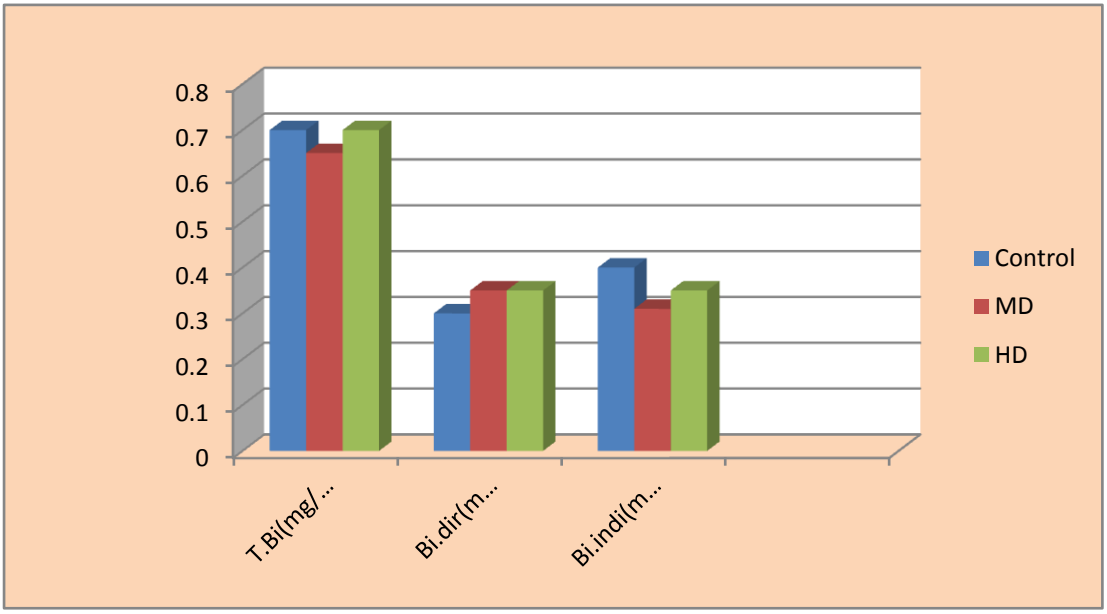


Chart 5 : The mean value of ALP,AST,ALT of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

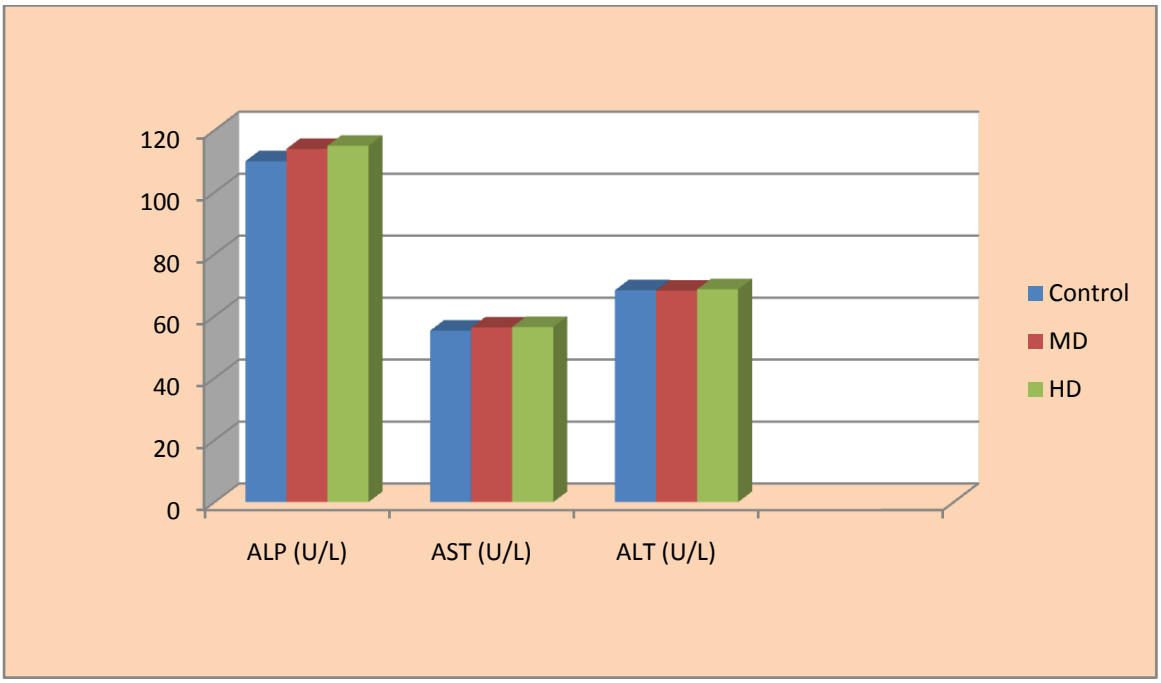


Chart 6: The mean value of Total protein , Albumin, Globulin of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

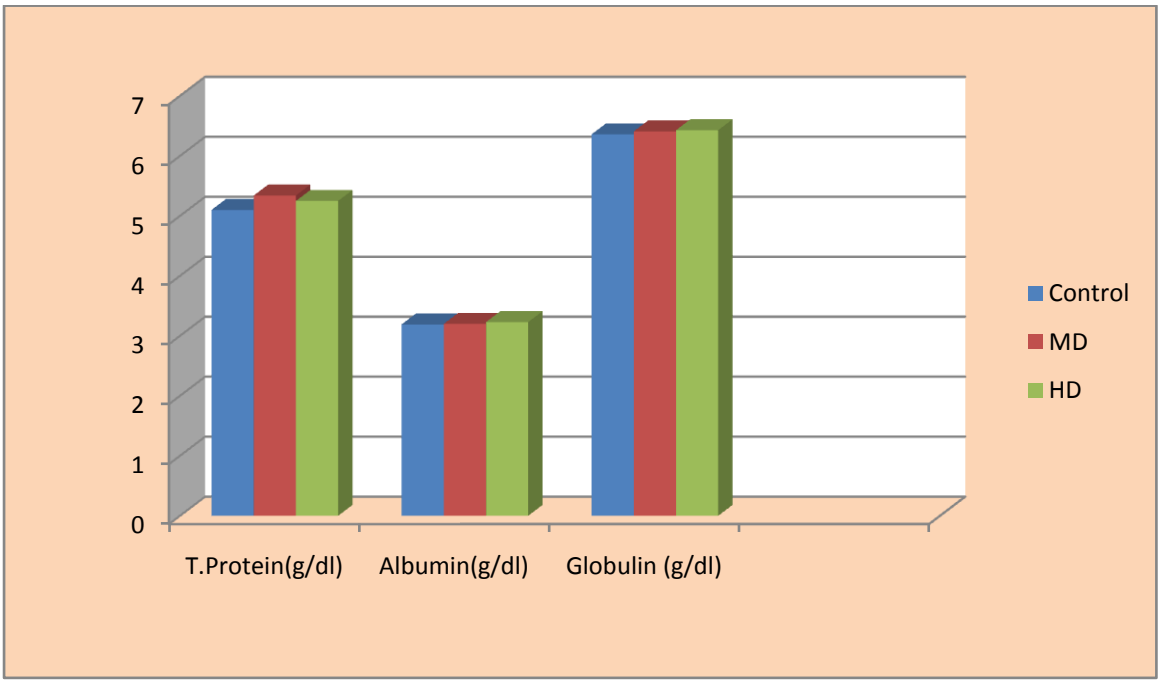


Table 16: Effect of treatment with Pattai Vallathagi on renal parameters:

Renal function test

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE
Urea(mg/dl)	15.23±0.47	15.11±0.11	15.99±0.11
Creatinine(mg/dl)	0.55±0.05	0.62±0.03	0.66±0.01
Uric acid(mg/dl)	2.35±0.22	2.22±0.20	2.27±0.14
Na m.mol	145.50±0.55	146.50±0.55	146.66±0.52
K m.mol	4.51±0.01	4.72±0.02	4.87±0.20
Cl m.mol	98.20±0.10	100.20±0.10	100.30±1.10

Values are mean of a 10animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

Chart 7 : The mean value of Urea of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

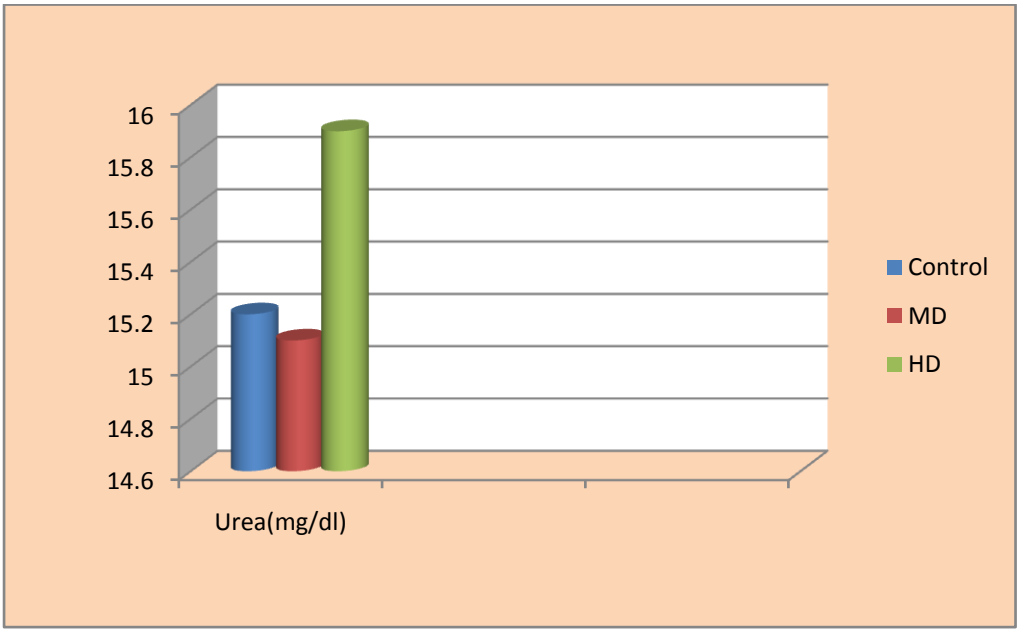


Chart 8 :The mean value of Creatinine, Uric acid of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

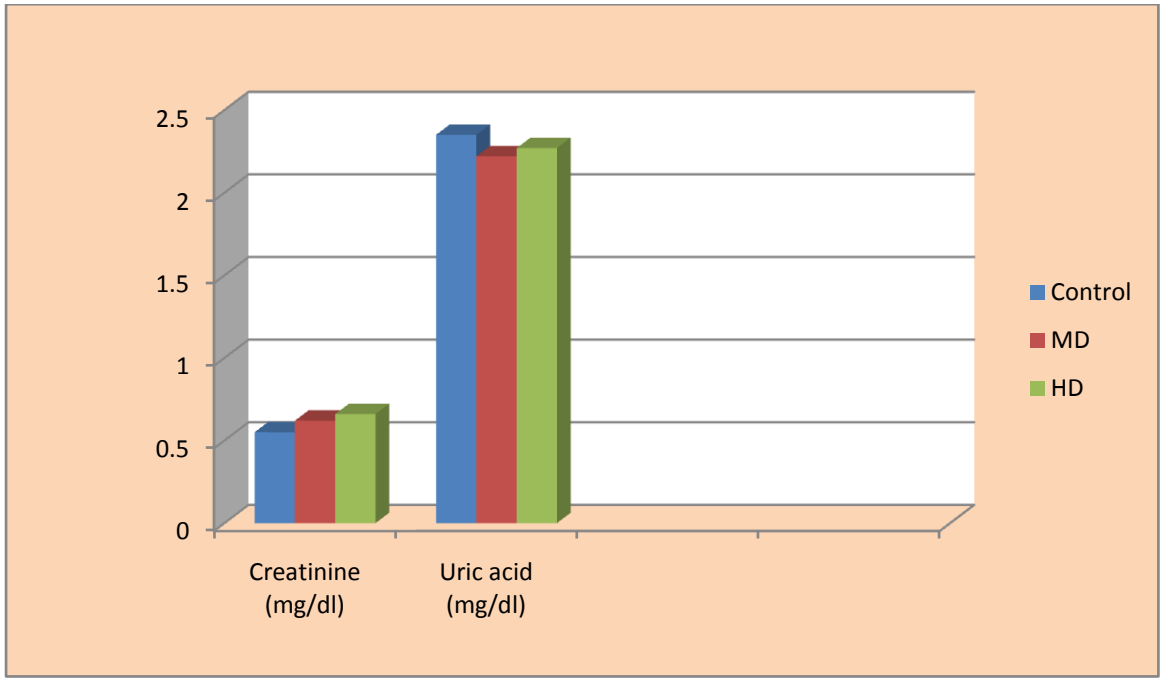


Chart 9 : The mean value of Na,K,Cl of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

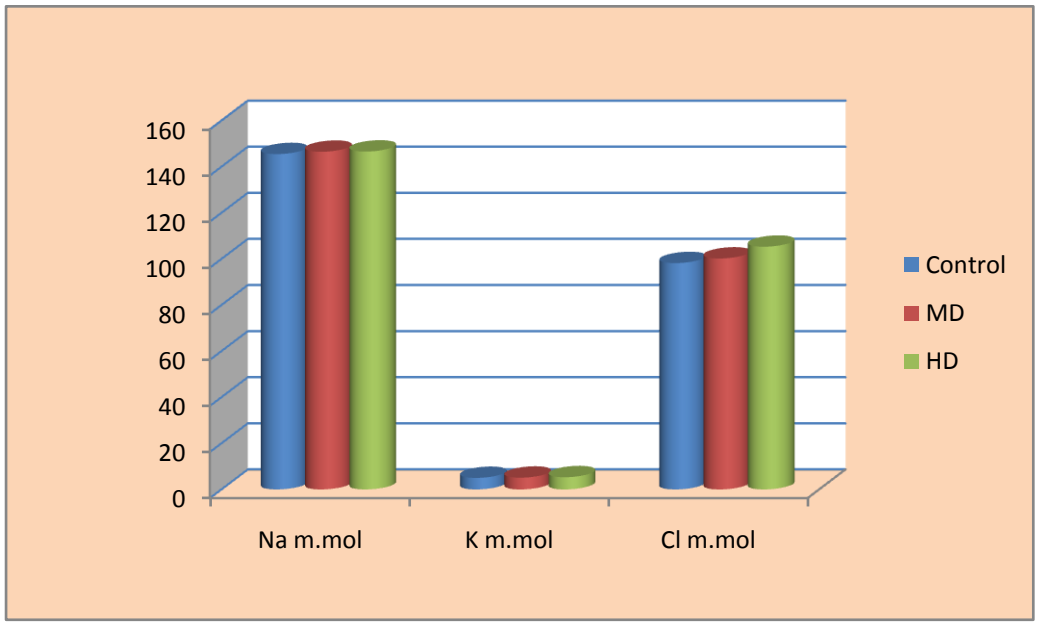


Table 17: Effect of treatment with Pattai Vallathagi on Lipid Profile

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE
Total cholesterol (mg/dl)	63.6±8.5	64.50±5.2	62.5±.8.7
HDL(mg/dl)	12.05±0.02	12.11±0.05	12.22±0.09
LDL(mg/dl)	35.83±0.42	36.73±0.36	37.18±0.70
VLDL (mg/dl)	15.80±0.03	15.81±0.02	15.72±0.04
Triglycerides(mg/dl)	78.33±0.52	76.0±1.26	76.35 ±0.82
TC/HDL ratio (g/dl)	3.18±0.04	3.11±0.04	3.22±0.03
Blood glucose (mg/dl)	121.90±0.66	121.84±0.82	121.98±0.36

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

Chart 10 : The mean value of T.Cho, HDL,LDL,VLDL.Triglycerides of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

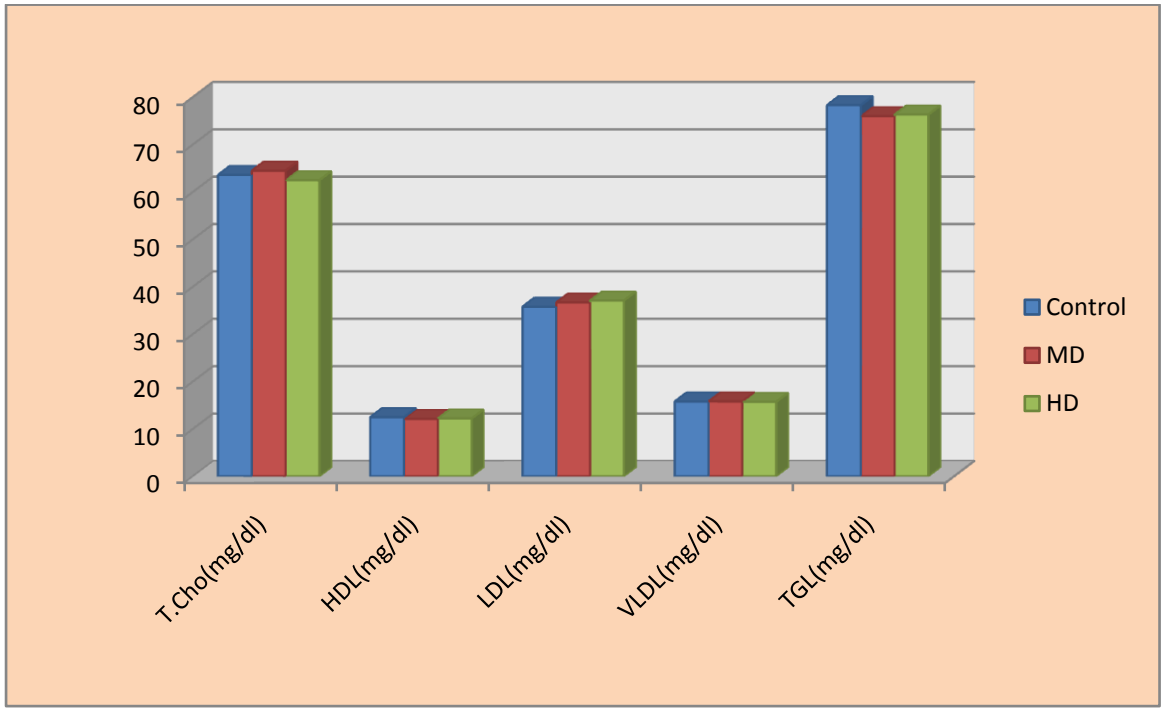


Chart 11 : The mean value of Blood Glucose of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 28 days

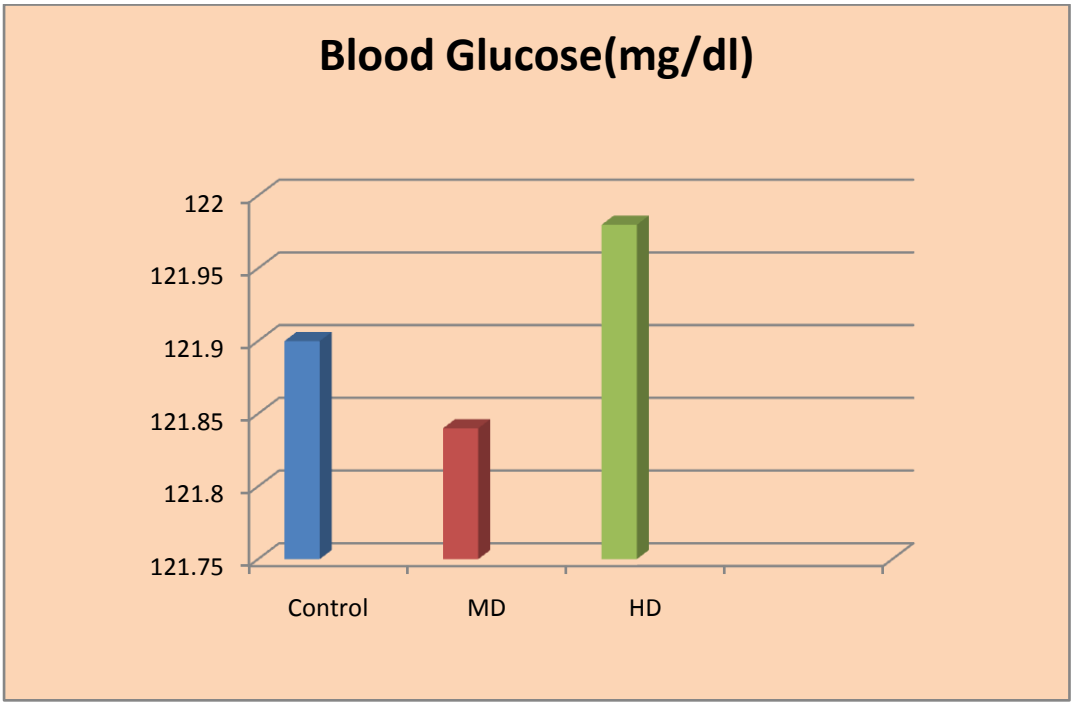


Table 18: Urine Analysis

PARAMETERS	CONTROL	MID DOSE	HIGH DOSE
Transparency	Clear	Slightly turbid	Slightly turbid
Specific gravity	1.010	1.010	1.010
PH	>7.0	>7.2	>7.2
Protein	Nil	Nil	Nil
Glucose	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve
Ketones	-ve	-ve	-ve
Blood	Absent	Absent	Absent
Urobilinogen	Normal	Normal	Normal
Pus cells	0-cells/HPF	0-cells/HPF	1-cells/HPF
RBC	Nil	Nil	1-cells/HPF
Epithelial cells	Nil	1-cells/HPF	Nil
Crystals	Nil	Nil	Nil
Casts	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen
Colour	Yellow	Yellow	Yellow

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* $p<0.05$;**$p<0.01$.N=10

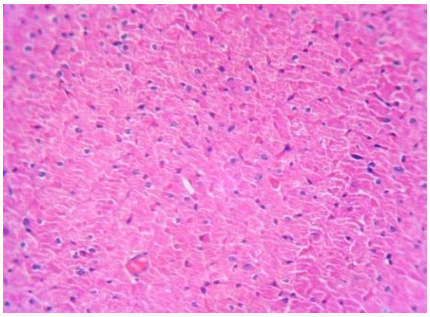
Table 19: Effect of Pattai Vallathagi on organ weight after 28 days treatment in rats

PARAMETER	CONTROL	MID DOSE	HIGH DOSE
Liver	4.51±0.44	4.03±0.39	4.50±041
Heart	1.05±0.10	1.18±1.13	1.1±0.11
Lung	0.83±020	0.93±0.09	0.90±0.08
Spleen	0.64±0.07	0.58±0.07	0.57±0.06
Ovary	1.69±0.14	1.78±0.15	1.78±0.18
Testes	1.48±0.10	1.66±0.15	1.69±0.15
Brain	1.56±0.15	1.56±0.14	1.56±0.14
Kidney	1.05+0.10	1.18+0.13	1.1+0.11
Stomach	1.36±0.12	1.38±0.11	1.38±0.11

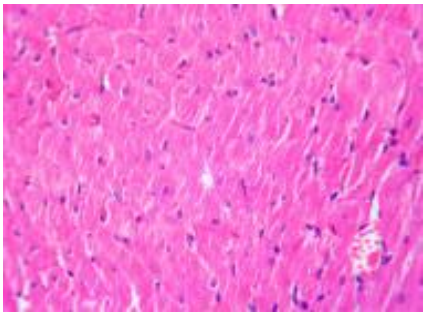
(Fig 2)HISTOPATHOLOGICAL STUDIES OF VARIOUS ORGANS AFTER THE 28 DAYS REPEATED DOSE ORAL TOXICITY STUDY OF PATTAI VALLATHAGI IN WISTAR ALBINO RATS

HEART

a. Control

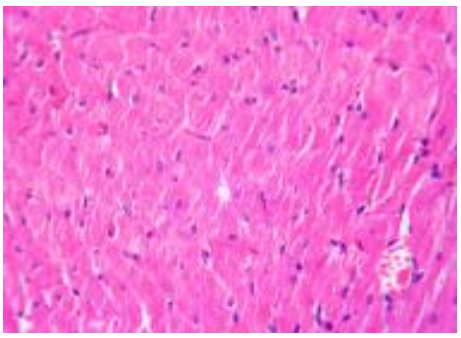


b. Mid Dose



c.

High Dose



Observations

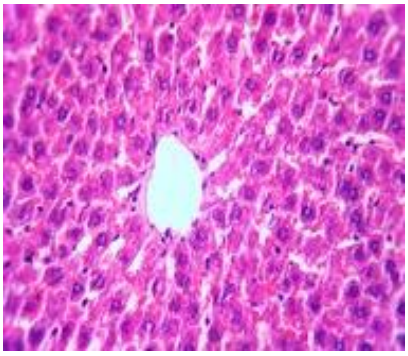
a) Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei.

b) Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei

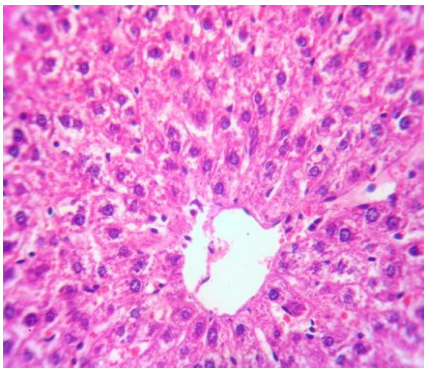
c) Section of the heart showed normal muscle fibres with acidophilic cytoplasm and centrally located nuclei

LIVER

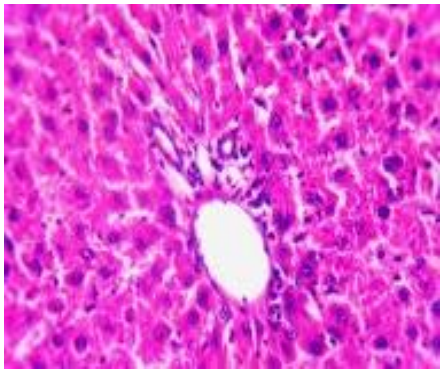
a. Control



b.Mid Dose



c. High dose

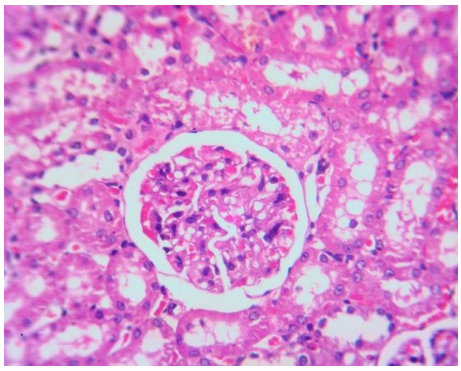


Observations

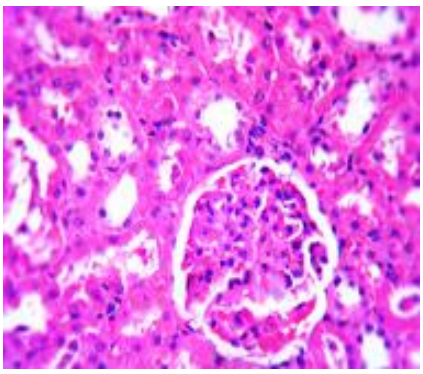
a) Section of liver from control animals showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract .
b) Section of liver showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract
c) Section of liver showed no degeneration of hepatocytes, focal steatosis, congestion of central vein and inflammation of portal tract

KIDNEY

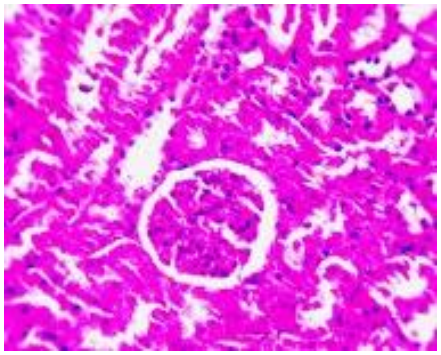
a.Control



b. Mid dose



c. High dose



Observations

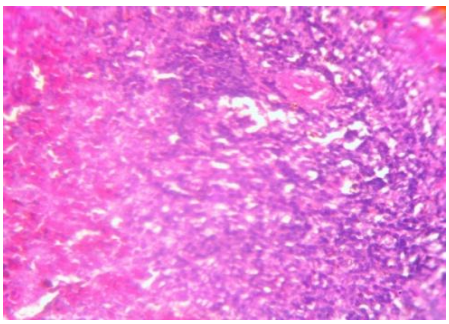
a) Section of kidney from control animals showed normal size of glomeruli with normal tubules.

b) Section of kidney showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney.

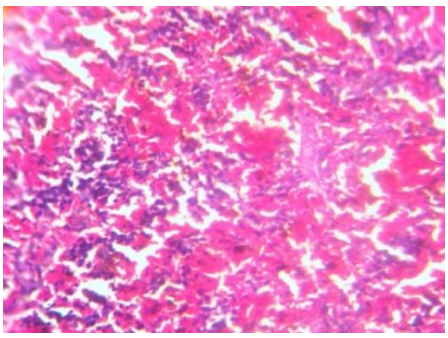
c) Section of kidney showed normal glomeruli and there is no necrosis of tubular epithelium in the kidney

SPLEEN

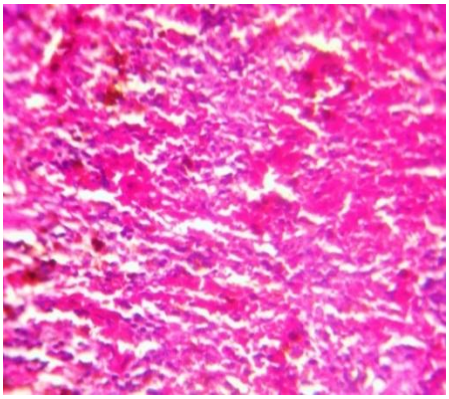
a. Contral



b.Mid dose



c.High dose



Observations

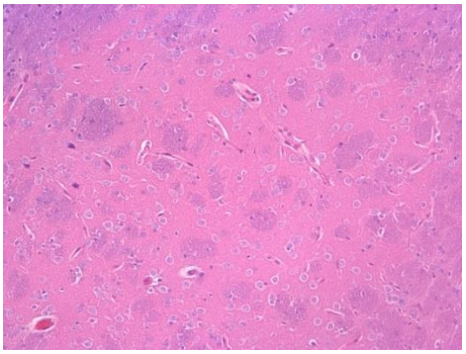
a) Section of spleen from control animal showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp.

b) Section of spleen showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp with normal structure.

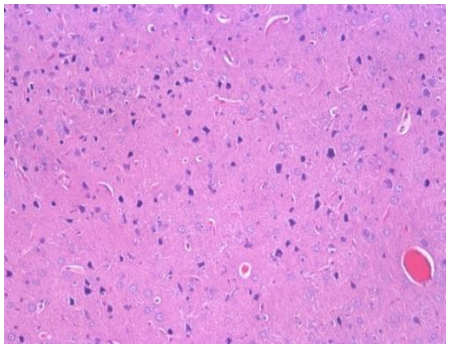
c) Section of spleen showed normal granular hemosiderin pigment predominantly within macrophages in the red pulp with normal structure

BRAIN

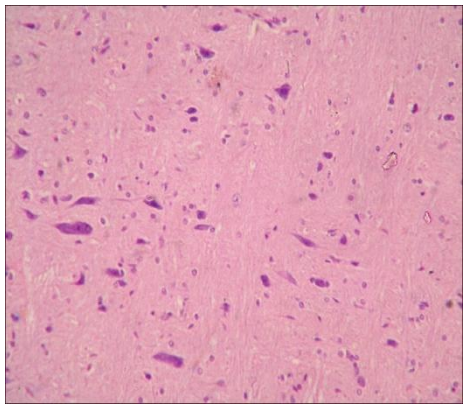
a. Control



b. Mid dose



c. High dose



Observation

a. Section of brain from control animals showed brain cortex with normal architecture.

b. Section of brain Treated animals showed brain cortex with normal architecture.

c. Section of brain Treated animals showed brain cortex with normal architecture

REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY

Results :

Sub-acute oral toxicity repeated dose of ***Pattai vallathagi*** on rats were conducted. All animals from the treated dose survived throughout the dosing period of 28 days. Various parameters were studied and the interpretation of the study result is discussed below.

Clinical signs:

No abnormal behavioural signs were observed during the study period.

Mortality

The test drug "*Pattai Vallathagi*" did not cause any mortality in mid and high dose levels and were considered as safe dose levels.

Body weight:

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited overall mild weight gain through out the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Haematological investigation interpretation

The haematological investigation results of the rats conducted on 28th day after the repeated dose of the drug revealed the values of different parameters. The increase and decrease in the values obtained were all within the normal biological and laboratory limits.

Biochemical investigation interpretation

The biochemical investigations were conducted on 28th day and the result is produced. The results revealed there is no significant changes in the values of different parameters with that of the control. All the values were within the normal biological and laboratory limits.

Histopathology interpretation :

The histopathological study of the organs such as heart, liver, spleen,kidney and Brain were normal in control, and all test groups.

90 DAYS REPEATED DOSE ORAL TOXICITY STUDY :

Table 20 : Body weight of wistar albino rats group exposed to *Pattai vallathagi* For 90 days

DAYS	Weight(gms)/Days				P value (p)*
	Control	Low dose	Mid dose	High dose	
1	162.4±29.65	162.29 ± 21.83	163± 13.09	162.5± 28.17	NS
15	161 ± 28.49	162.8 ± 23.54	171.7 ± 29.83	179.8 ± 32.13	NS
30	184.4 ± 28.83	184.71 ± 14.76	182.8 ± 32.17	190.2 ± 28.55	NS
45	202.7± 27.81	205.6± 29.12	203.1± 19.07	207.9± 22.05	NS
60	226.6±33.47	232.6±23.91	230.6±33.61	226.6±23.63	NS
75	240.8 ± 26.76	244.8 ± 28.08	242.0± 28.70	240.8 ±26.87	NS
90	263.5± 27.94	266.9± 27.68	268.2± 27.31	266.1± 29.41	NS

NS- Not Significant, **($p < 0.01$),*($p < 0.05$), n =6 values are mean ± S.D (One way ANOVA followed by Dunnett's test

Table 21: Haematological parameters of Wistar albino rats group exposed to *Pattai Vallathagi* for 90 days (sub chronic toxicity study)

Parameter	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	12.88±0.54	12.83±0.38	13.48±0.98	12.96±0.50	N.S
Total WBC (cells/cu.mm)	11.2±4.6	10.9±6.6	11.5±4.6	11.3±3.3	N.S
Platelets cells/ul	353.2±0.32	339.1±0.23	366.1±0.23	377.3±0.08	N.S
Total RBC (cells/cu.mm)	7.33±0.21	7.08±0.19	7.33±0.33	7.11±0.45	N.S
PCV%	38.62±1.60	38.49±1.17	40.48±2.94	38.81±1.60	N.S
MCV (ft)	89.16±2.71	91.66±3.55	89.33±3.72	92.66±2.87	N.S
MCH(pg)	33.5 ± 3.01	30.5 ± 3.83	32.16 ± 3.48	30.66 ± 1.03	N.S
MCHC (gm/dl)	33.33±2.50	36.5±1.87	35±2.36	36±4.77	N.S

N.S- Not Significant, **($p < 0.01$), *($p < 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 12: The mean value of HB, Total RBC, Total WBC of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 90 days

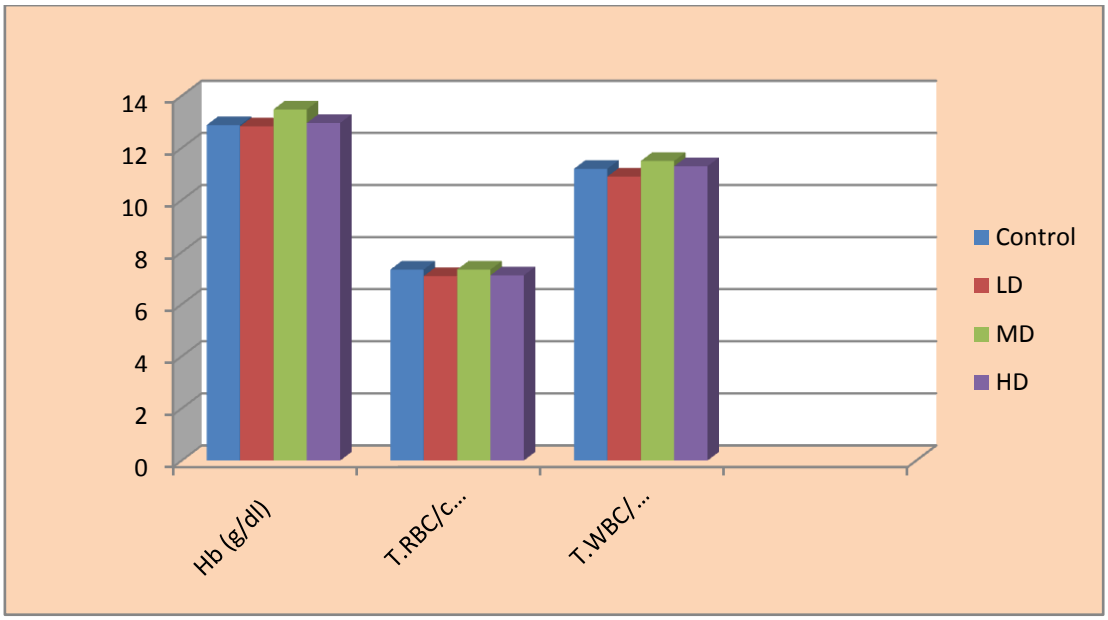


Chart 13: The mean value of Platelets of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 90days

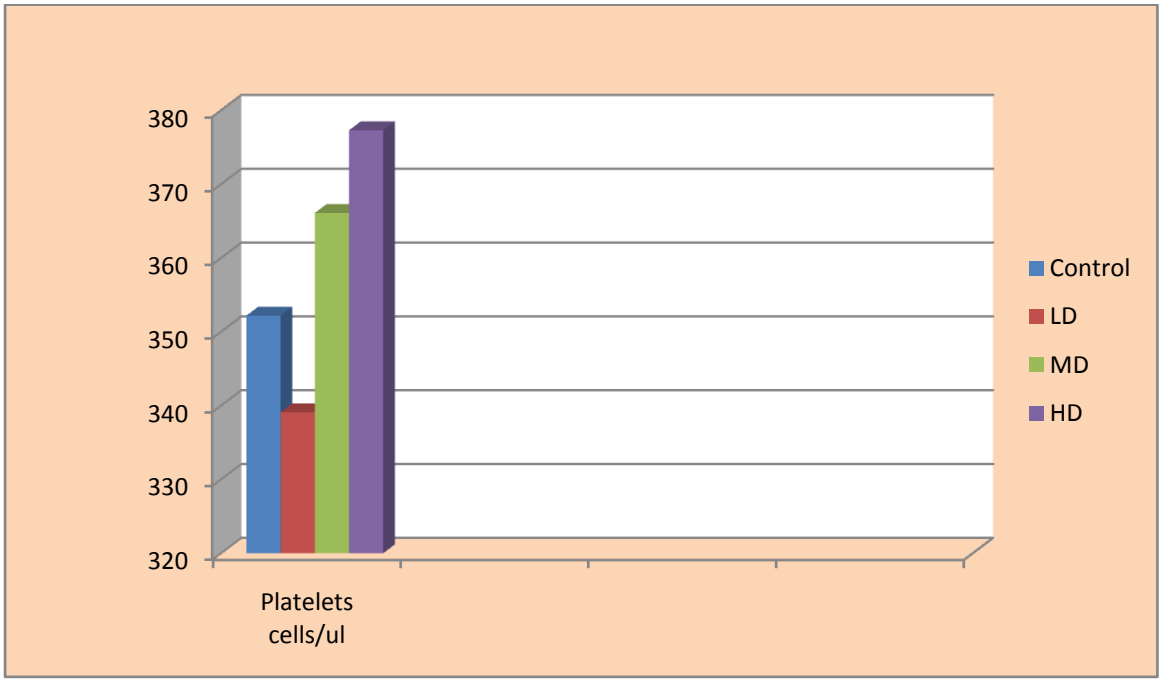


Chart 14 : The mean value of PCV,MCV,MCH,MCHC of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 90 days

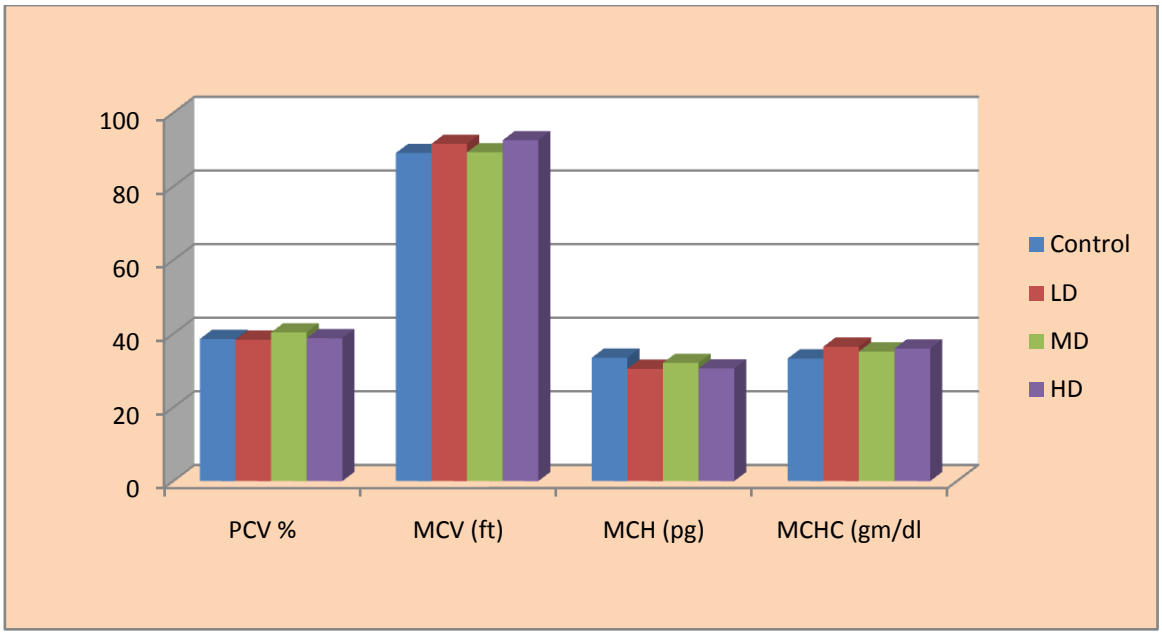


Table 22 : Blood sugar test of Wistar albino rats group exposed to Pattai Vallathagi for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Bl.sugar (mg/dl)	124.66±7.31	123.66±11.62	113.92±2.87	119.12±4.41	N.S.

N.S- Not Significant, **(p < 0.01), *(p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 15: The mean value of Blood sugar of control and treated groups of wistar albino rats exposed to Pattai vallathagi for 90 days

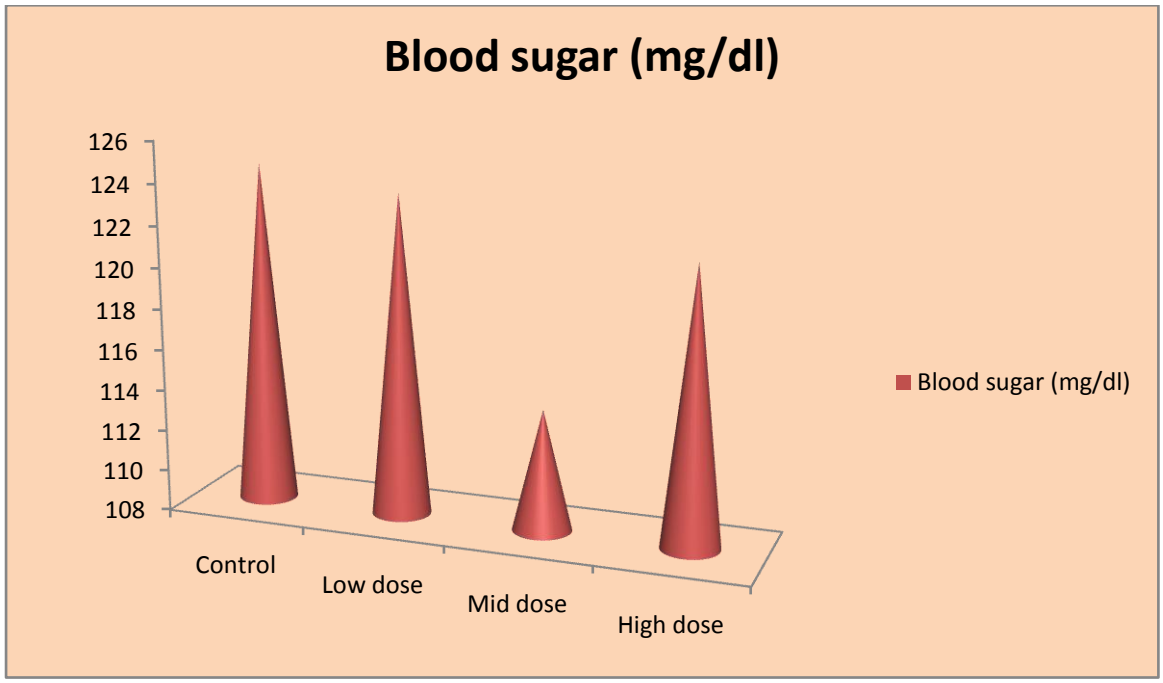


Table 23: Renal function test of Wistar albino rats group exposed to Pattai Vallathagi for 90days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	29±6.32	27.66±7.71	23.83±6.08	28.83±7.19	N.S
CREATININE(mg/dl)	0.58±0.19	0.73±0.12	0.45±0.10	0.58±0.20	N.S

NS- Not Significant, **(p < 0.01), * (p <0.05) , n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 16: The mean value of Urea of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 90 days

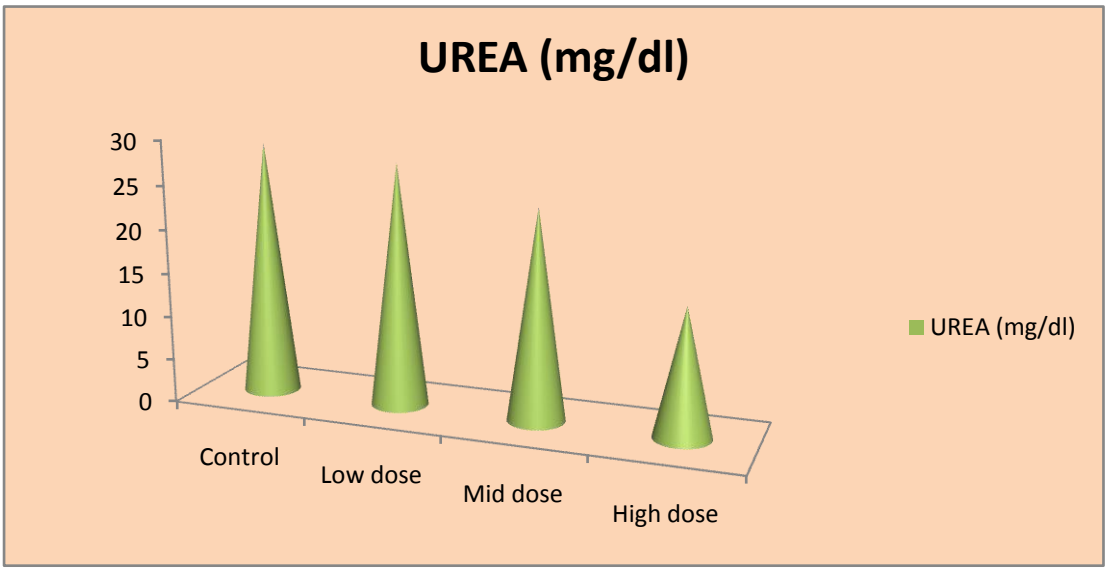


Chart 17: The mean value of Creatinine of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 90 days

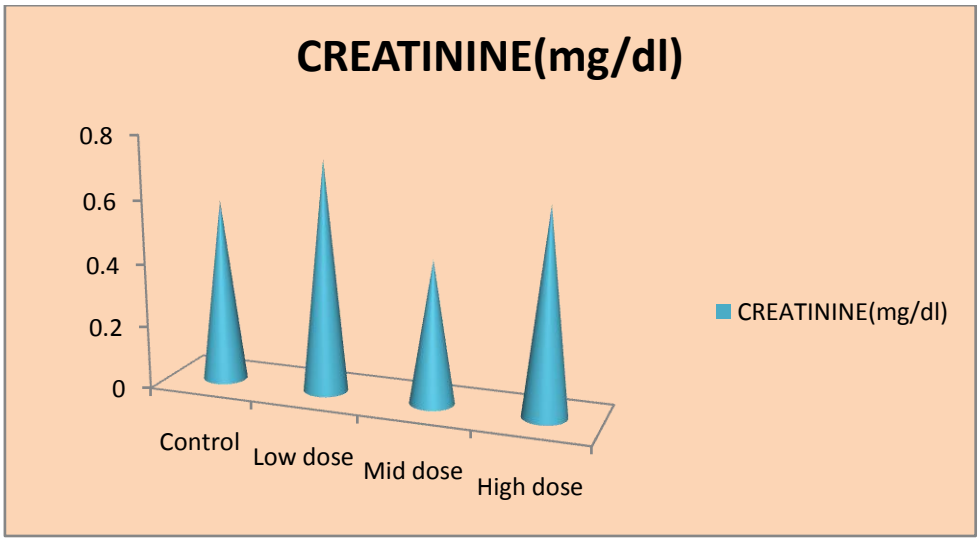


Table 24: Sub chronic toxicity - Lipid profile test of Wistar albino rats group exposed to Pattai Vallathagi for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
Tcho(mg/dl)	119.16±5.34	119.6±6.53	116.56±10.41	120.33±4.88	N.S
HDL(mg/dl)	41.83±3.65	46±3.16	47.64±5.08	39.16±4.21	N.S
LDL(mg/dl)	49.33±6.43	48.83±6.79	39.29±10.16	52.66±5	N.S
VLDL(mg/dl)	27.93±2.27	25.06±1.04	27.13±4.05	28.4±1.62	N.S
\TGL(mg/dl)	139.66±11.37	125.33±5.24	135.66±20.27	142±8.12	N.S

N.S- Not Significant, **(p <0.01), *(p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 18: The mean value of Lipid profile of control and treated groups of wistar albino rats exposed to Pattai vallathagi for 90 days

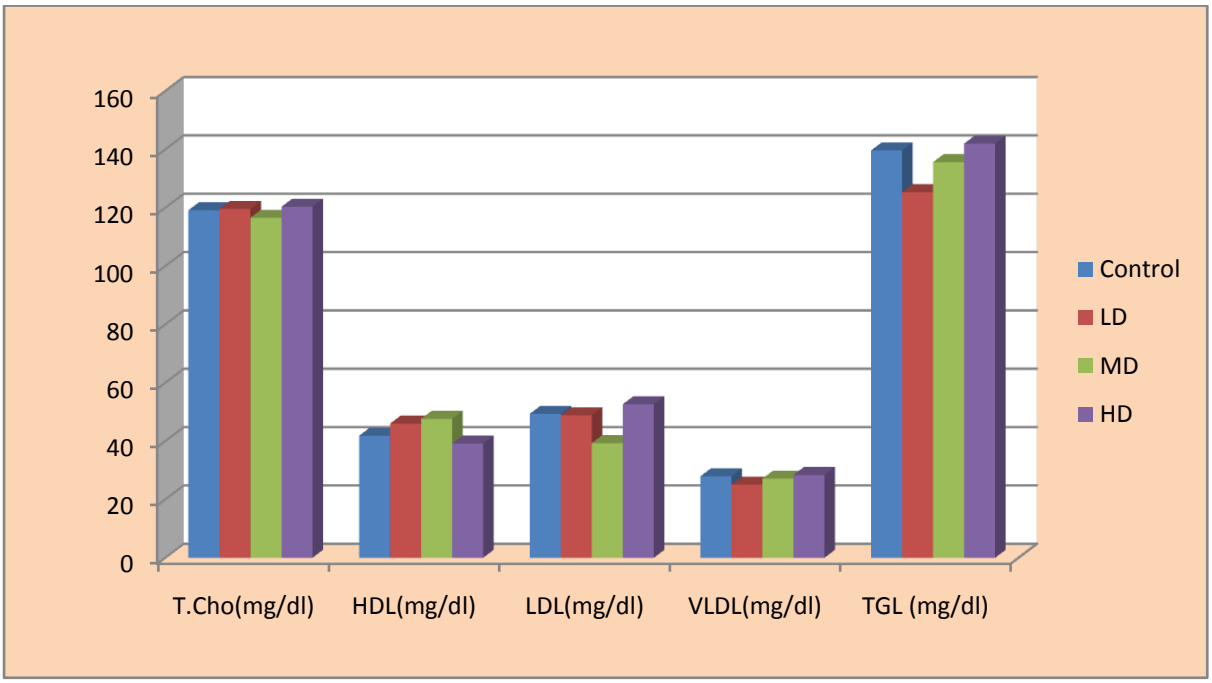


Table 25: Liver Function Test of Wistar albino rats group exposed to Pattai Vallathagi for 90 days

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T.BILIRUBIN(mg/dl)	0.68±0.17	0.63±0.15	0.76±0.103	0.68±0.07	N.S
SGOT(U/L)	24.16±6.90	22.5±4.84	28.33±3.01	23.5 ± 6.59	N.S
SGPT(U/L)	25.33±9.85	28.83±5.19	26.5±3.27	22.66±3.98	N.S
ALP(U/L)	63±8.07	77.33±17.03	69.16±16.24	63.33±6.08	N.S

NS- Not Significant, **(p <0.01), * (p <0.05), n = 6 values are mean ± S.D (One way ANOVA followed by Dunnett's test)

Chart 19 : The mean value of T.Bilirubin of control and treated groups of wistar albino rats exposed to Pattai vallathagi for 90 days

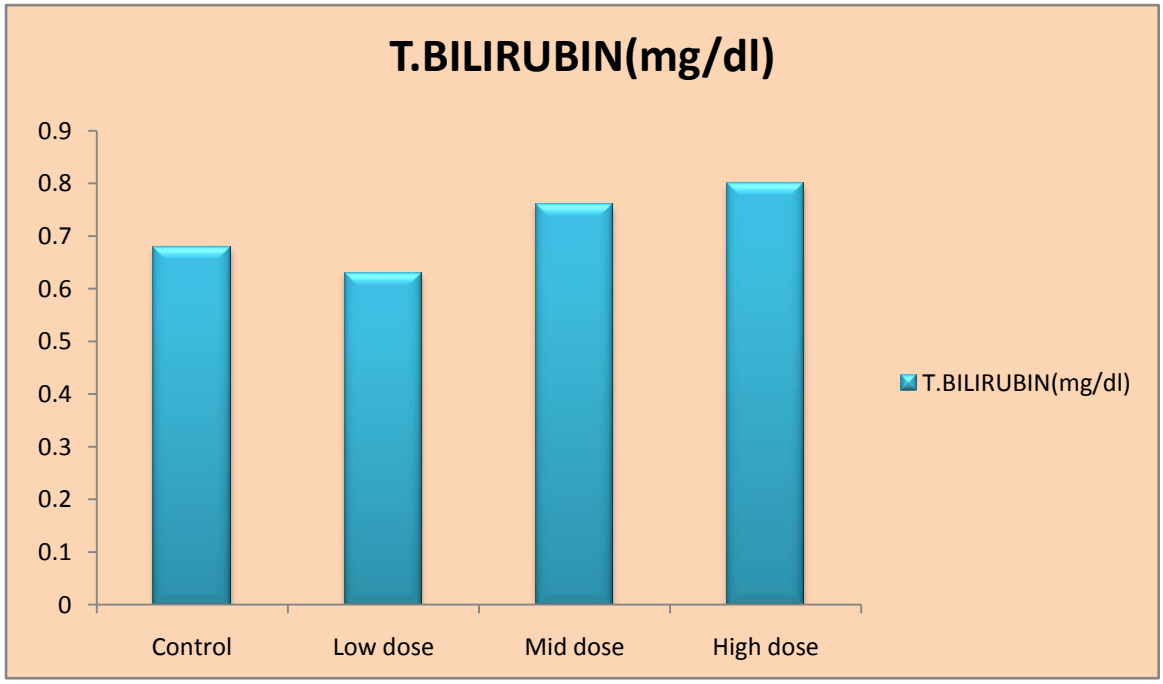
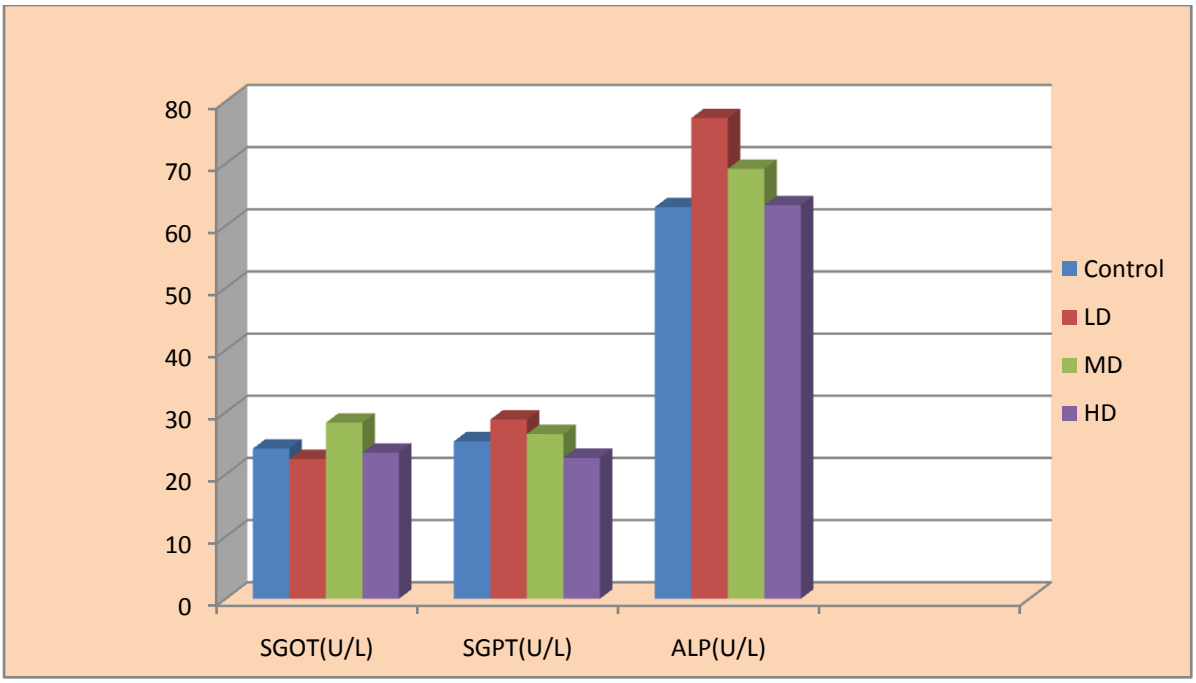
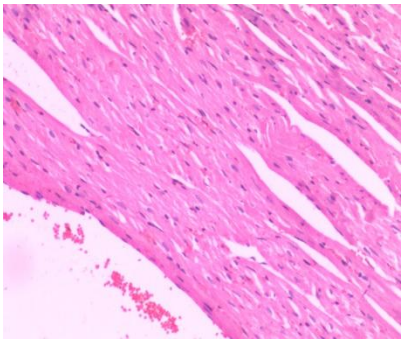


Chart 20: The mean value of SGOT, SGPT,ALP of control and treated groups of wistar albino rats exposed to Pattai Vallathagi for 90 days

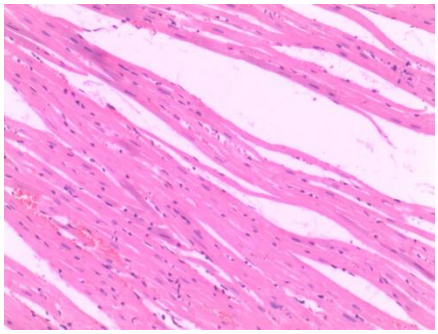


(Fig 3)HISTOPATHOLOGICAL STUDIES OF VARIOUS ORGANS AFTER THE 90 DAYS REPEATED DOSE ORAL TOXICITY STUDY OF PATTAI VALLATHAGI IN WISTAR ALBINO RATS

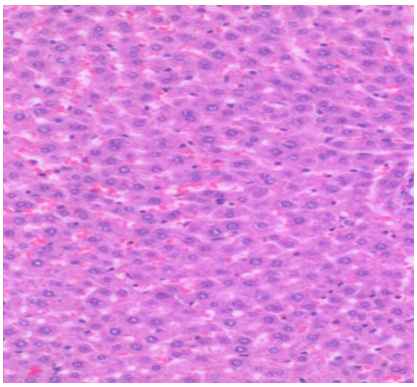
Control - Heart



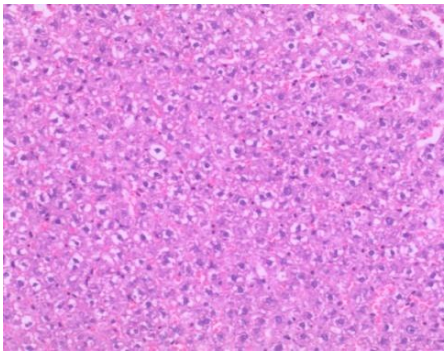
High dose - Hear



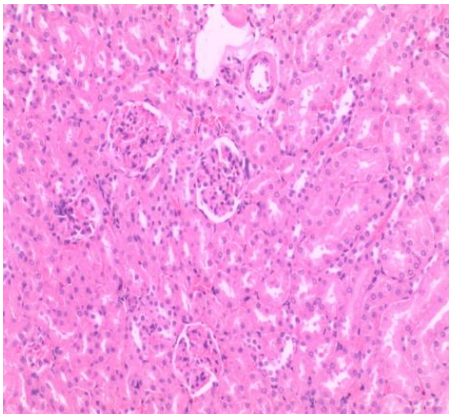
Control – Liver



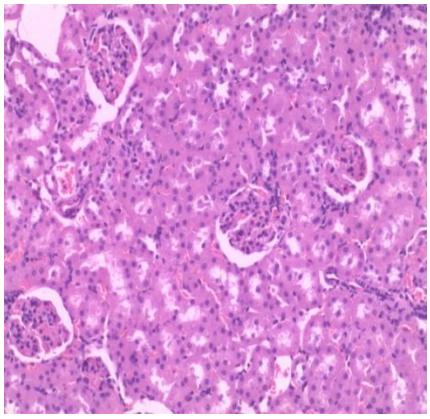
High dose - Liver



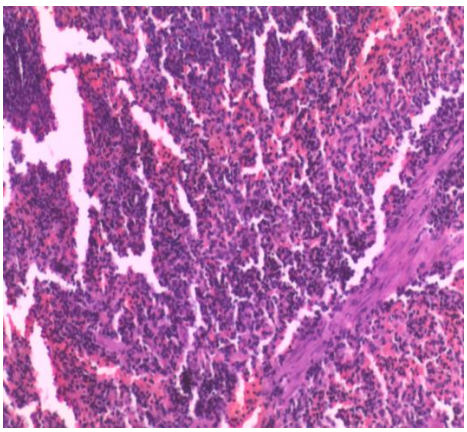
Control - kidney



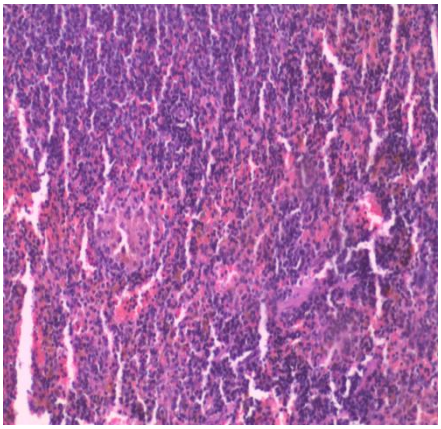
High dose - Kidney



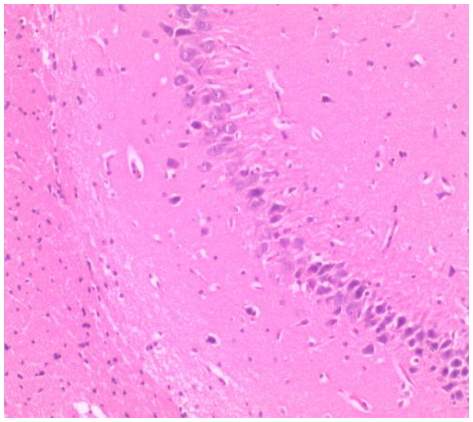
Control –Spleen



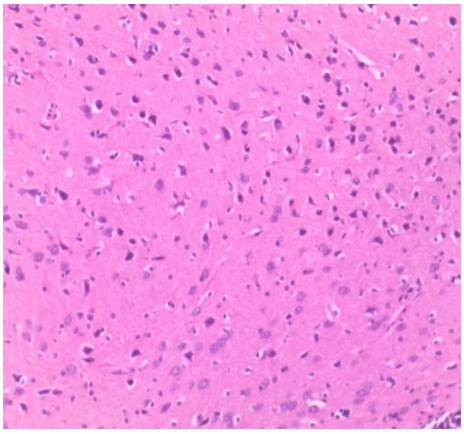
High dose - Spleen



Control – Brain



High dose - Brain



Observation :

Heart

- Atrial and ventricular wall of both the heart sample appears normal
- Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear central arrangement.

Liver

- Appearance of portal vein, bile duct and hepatic artery was normal
- Hepatocellular architecture, including hepatic sinusoid and hepatic cord was normal.

Kidney

- Glomerular cell integrity, basement membrane and nephrotic bundle appears normal
- No signs of lesion or inflammation were observed
- Proximal and distal convoluted tubule appears normal

Spleen

- Appearance of LF – lymphoid follicle; PALS – periarterial lymphoid sheath was normal with no significant signs of enlargement
- Presence of marginal at the interface of the red pulp with the PALS and follicles was observed

Brain

- Arrangement of the neurons appears intact with no sings of degeneration or apoptotic changes in both the samples
- Cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm.

RESULTS OF REPEATED DOSE 90 DAYS ORAL TOXICITY STUDY :

Repeated dose 90 days oral toxicity study of *Pattai Vallathagi* on rats were conducted. All animals from the treated dose survived throughout the dosing period of 90 days. No abnormal behavioural signs were observed during the study period.

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited over all mild weight gain throughout the dosing period of 90 days.

The haematological and bio chemical investigations were conducted on 91th day after the repeated dose of the drug revealed there is no significant changes in the values of different parameters with that of the control.

Histopathological study of the organ such as heart, kidney, liver, spleen and Brain were normal in control and test groups.

Table 26 : Pharmacological analysis :Effect of pattai Vallathagi on cotton pellet induced granuloma model

Groups	Treatment	Mean wet weight of pellet(mg)	Percentage inhibition	Mean dry weight of pellet(mg)	Percentage inhibition
I	Control	195.50±2.17	0	45.60±1.39	0
II	Dexamethasone(0.5mg/kg)	92.33±3.46**	52.77	20.88±0.72**	54.21
III	Pattai vallathagi(200mg/kg)	170.33±3.61*	12.88	35.63±1.02*	19.86
IV	Pattai vallathagi(400mg/kg)	135.16±4.07**	30.86	30.68±0.28**	26.56

N= 6, values are expressed as mean± SEM ,P<0.01 when compared with control. The results were analyzed by ANOVA followed by Dunnet's test (P value less than 0.01 was considered as statistically significant

Chart 21 :Effect of pattai Vallathagi on the mean wet of cotton pellet induced granuloma model

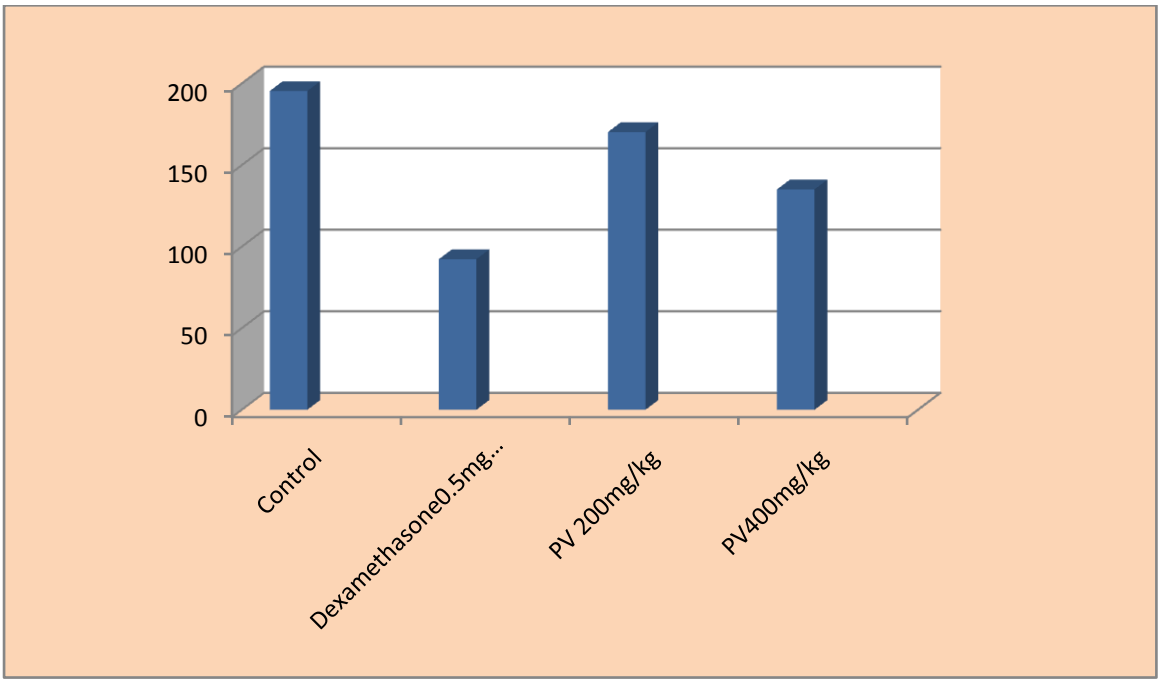


Chart 22 :Effect of pattai Vallathagi on percentage inhibition on wet cotton pellet induced granuloma model

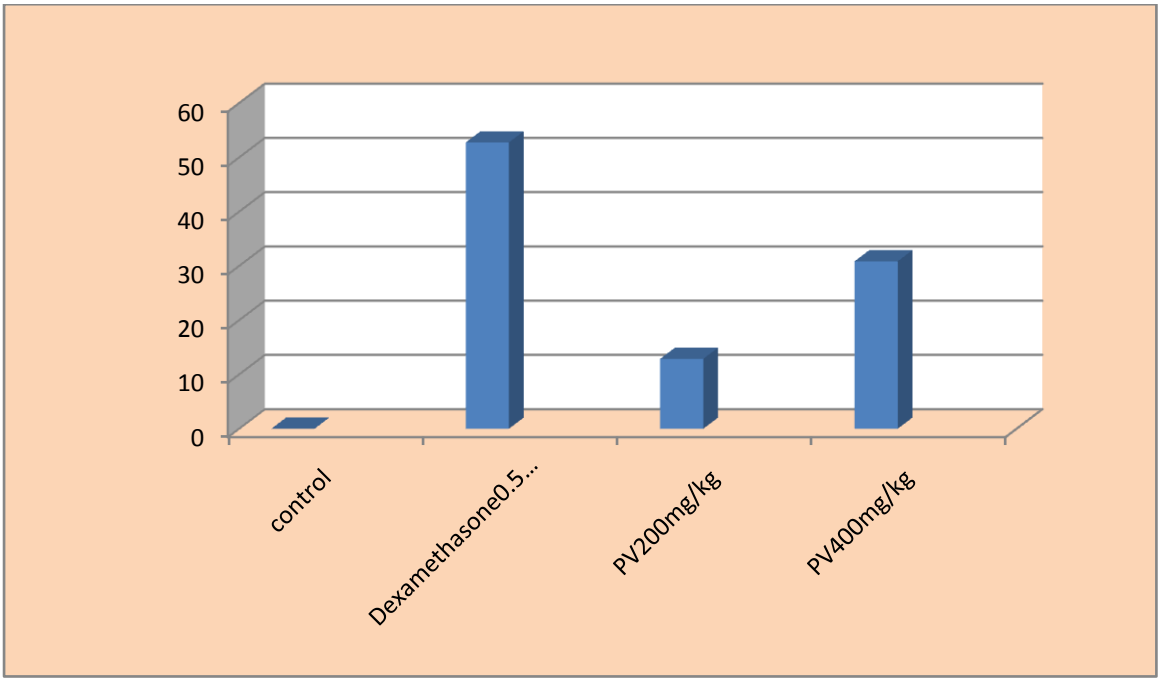


Chart 23 :Effect of pattai Vallathagi on mean dry of cotton pellet induced granuloma model

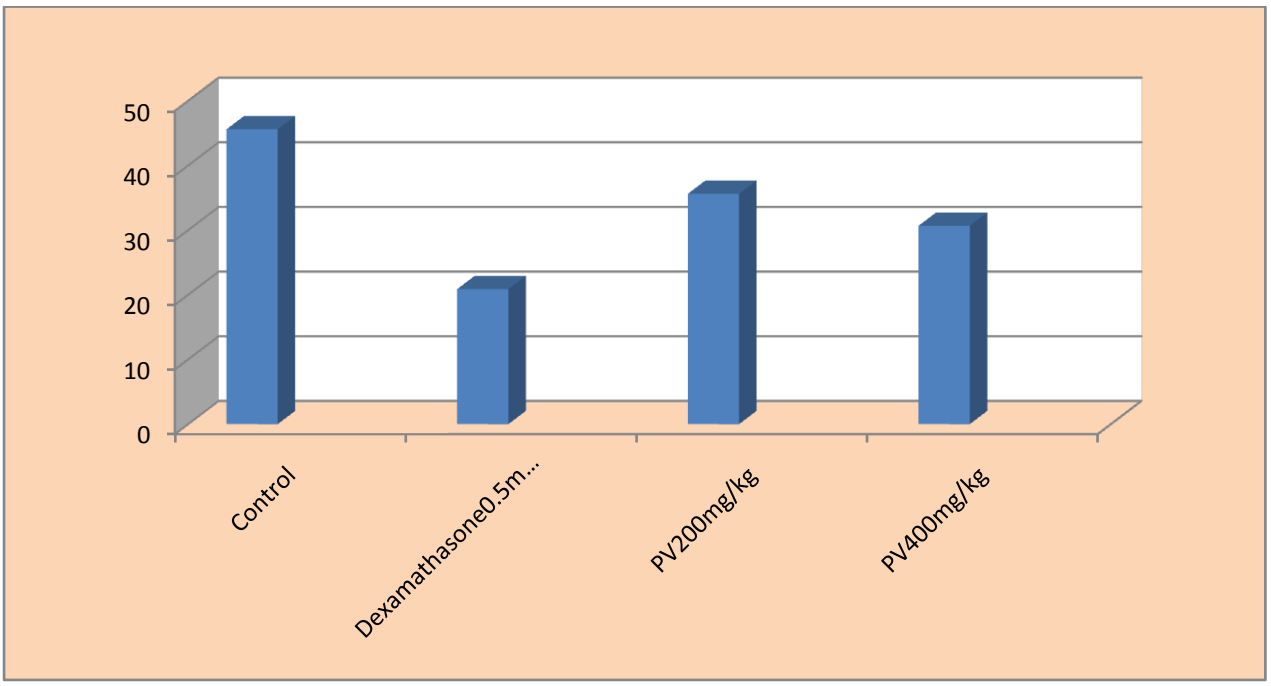
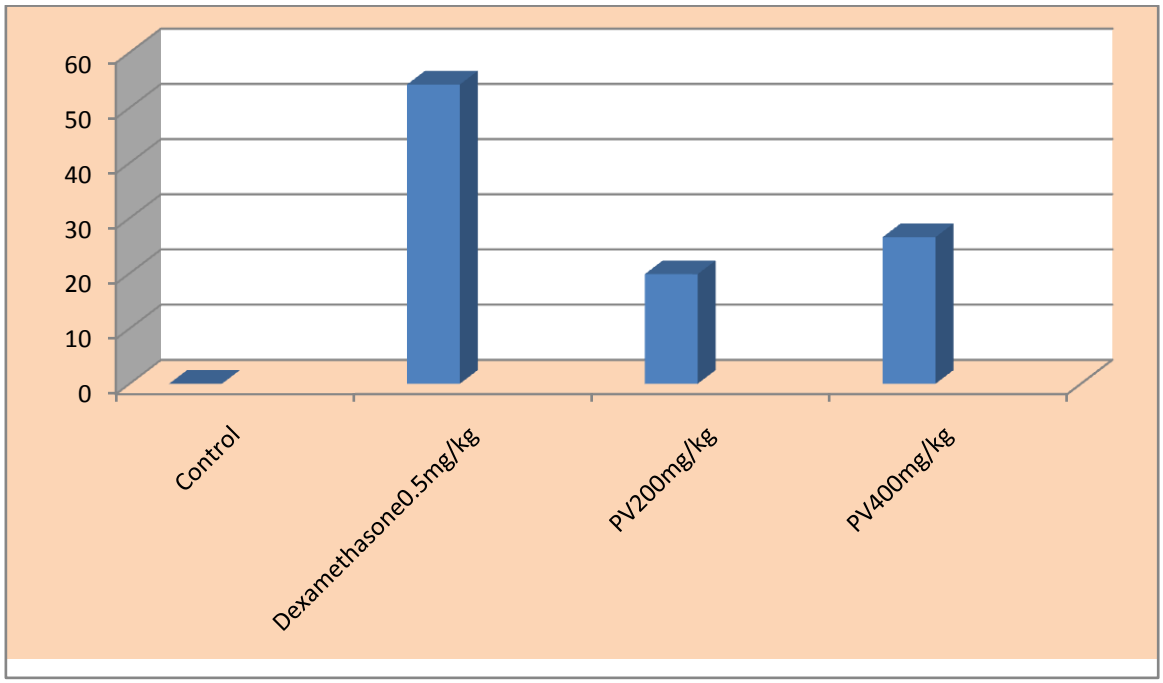


Chart 24 : Effect of pattai Vallathagi on percentage inhibition on dry cotton pellet induced granuloma model



RESULTS

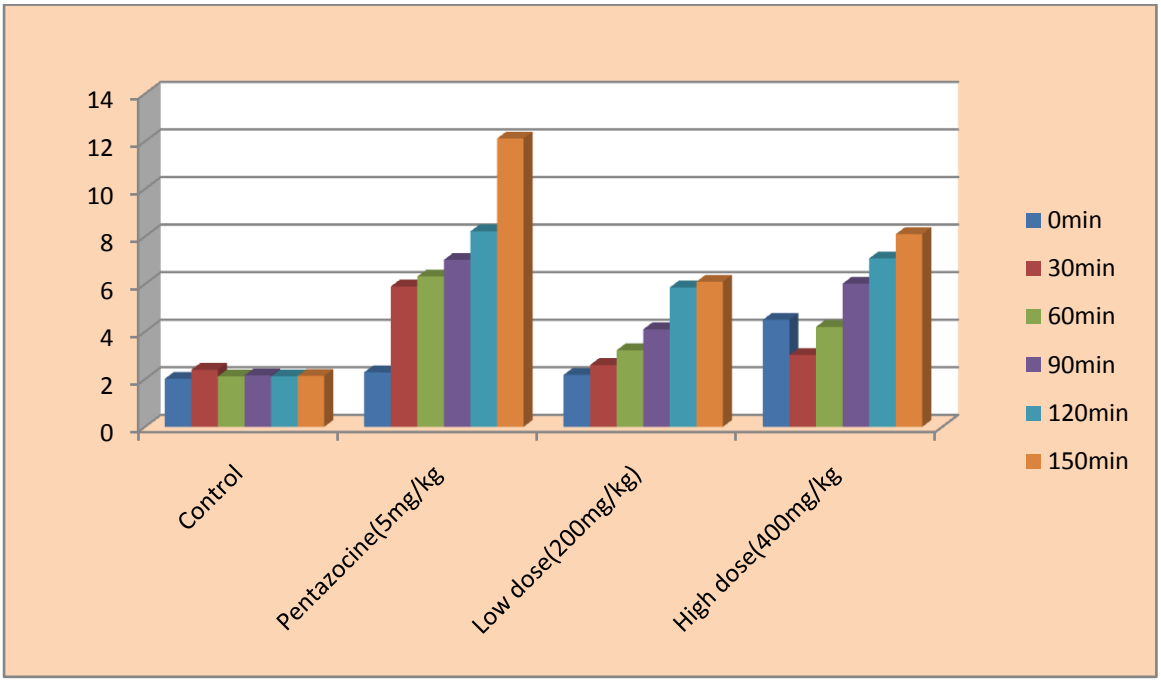
The results indicate that *Pattai vallathagi* at the dose level of 200mg/kg and 400mg/kg produced a decrease in wet granuloma weight 170.33±3.61* (12.88% inhibition) and 135.16±4.07**(30.86% inhibition) respectively when compared with control . Similarly there was a significant decrease in dry granuloma weight 35.63±1.02*(19.86% inhibition) and 30.68±0.28**(26.56% inhibition) respectively when compared with control. Among the two doses 400 mg/kg showed slightly lower reduced weight of granuloma than standard drug which was showed in table 26. Thus it was concluded that administration of *Pattai Vallathagi* at the dose of 400 mg/kg exhibited significant ($p<0.01$) anti -inflammatory activity in Cotton pellet granuloma model of inflammation in rats.

Table 27 : Pharmacological analysis – Analgesic activity of Pattai Vallathagi in Swiss albino mice

Groups	Treatment	Reaction time in sec					
		0min	30min	60min	90min	120min	150min
I	Control	2.02±0.16	2.04±0.10	2.12±0.10	2.16±0.16	2.12±0.12	2.14±0.12
II	Pentazocine (5mg/kg)	2.29±0.17	5.09±0.12*	6.32±0.06**	7.02±0.10**	8.22±0.14**	12.12±0.01**
III	Low dose (200mg/kg)	2.18±0.02	2.60±0.04	3.22±0.08	4.10±0.12*	5.86±0.12*	6.10±0.08*
IV	High dose 400mg/kg).	2.24±0.21	3.02±0.01	4.20±0.04*	6.02±0.15**	7.08±0.18**	8.10±0.02*

N= 6, values are expressed as mean± SEM ,P<0.05,P<0.01 when compared with control. The results were analyzed by ANOVA followed by Dunnet's test (P value less than 0.05,0.01 was considered as statistically significant

Chart 25 :Analgesic activity of Pattai Vallathagi in Eddy's Hot plate method



Result of Analgesic activity of *Pattai Vallathagi* in swiss albino mice

Analgesic activity was carried out by Eddy's Hot plate method. *Pattai Vallathagi* at the two doses 200 mg/kg showed significant ($p<0.05$) analgesic activity at reaction time 90 min (4.10±0.12*) and 400 mg/kg showed significant ($p<0.01$) analgesic activity at 120 min (7.08±0.18**) was slightly lower than the standard drug Pentazocine 8.22±0.14**.

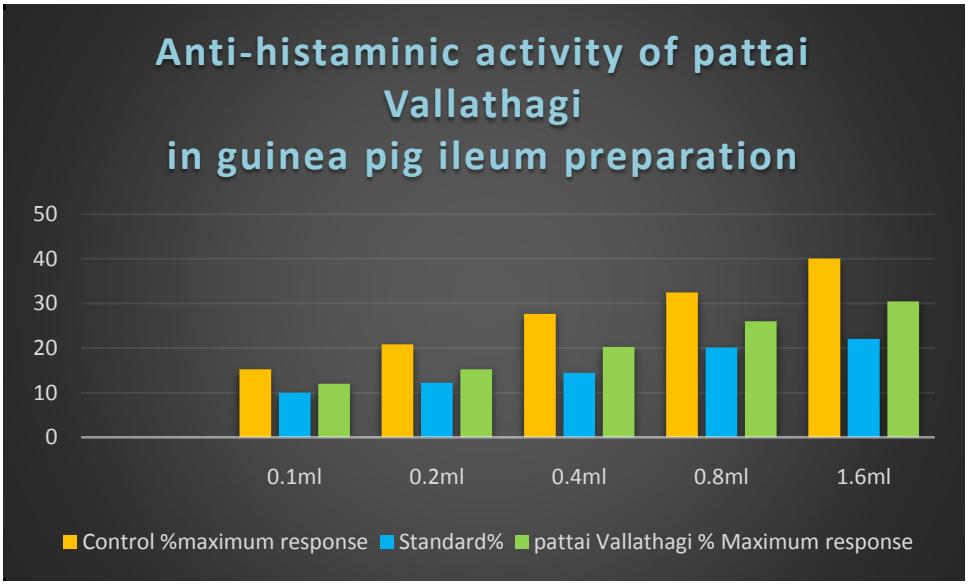
From these results it was obvious that *Pattai Vallathagi* has significant analgesic activity.

Table28 : Pharmacological analysis -Antihistaminic activity of pattaiVallathagi using guinea pig ileum preparation

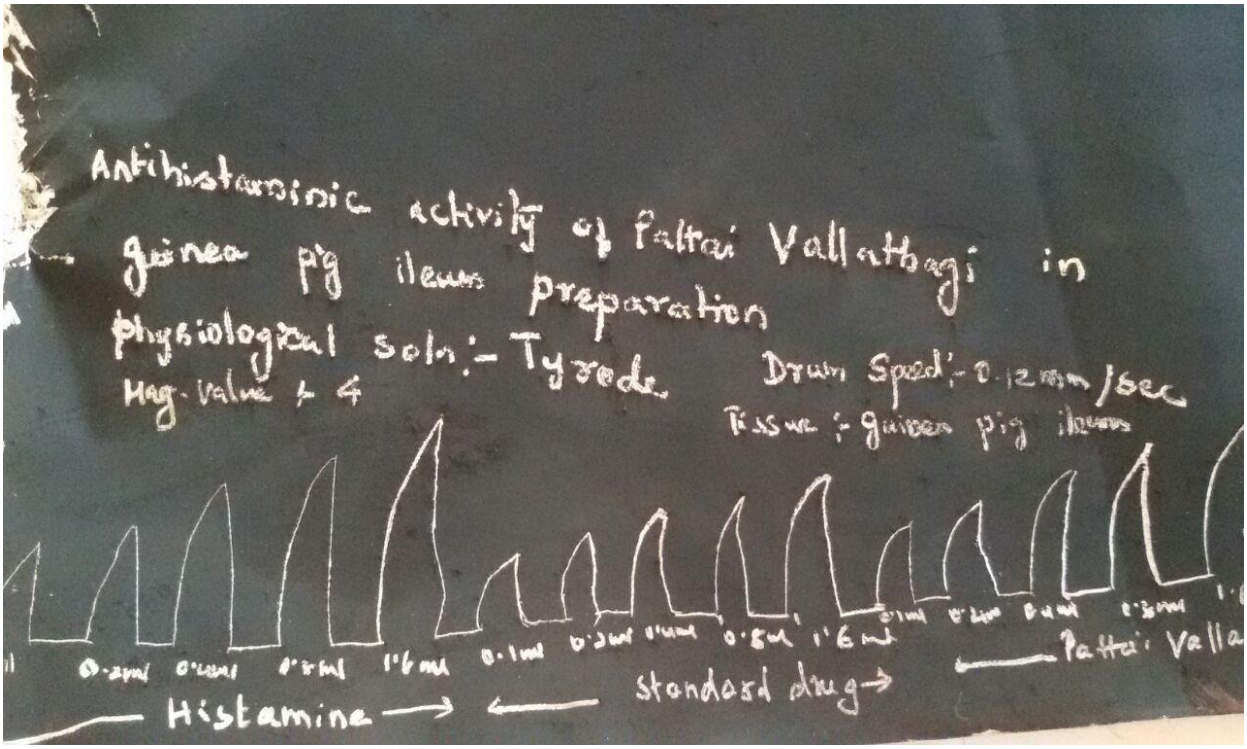
S.No	Dose of histamine	Log molar concentration of Histamine	Control %maximum response	Standard% Maximum response	pattai Vallathagi % Maximum response
1	0.1ml	7.08	15.20±1.01	10.01±1.46**	12.02±0.21**
2	0.2ml	6.79	20.82±1.06	12.21±2.01**	15.20±1.22**
3	0.4ml	6.48	27.62±1.22	14.45±1.04**	20.21±1.46**
4	0.8ml	6.18	32.42±1.26	20.12±2.31**	26.01±2.02**
5	1.6ml	5.88	40.02±1.22	22.04±1.04**	30.42±1.01**

Values are expressed as mean±SEM (n=6).**P<0.01 when compared to control group.

Chart 26 : Anti-histamine activity of *Pattai Vallathagi* in ileum cut terminal method



Graph : Pharmacological analysis – Anti- histamine activity of Pattai Vallathagi in albino guinea pig



Result of anti- histamine activity :

Histamine was released from mast cells and basophiles by antigenic stimulation causing smooth muscle contraction, increased vascular permeability and mucus formation. Mast cells with their mediator can be regarded as centre for initiation and mediation of early phase of allergic reaction and may be responsible for initiation of chronic allergic reaction.The stimulation of H_1 receptors produces graded dose related contraction of isolated guinea pig ileum.In the present study the trial drug *Pattai Vallathagi* significantly inhibited the histamine induced contraction of isolated guinea pig ileum preparation,indicating Anti-histamine activity

DISCUSSION

9.DISCUSSION

The drug *Pattai vallathagi* was selected from the Siddha literature ***"Siddha anuboga vaithiya navaneetha thirattu (part -10)"*** to validate the safety and its pharmacological activities (anti-inflammatory, analgesic and anti- histamine) in animal model.

The ingredients of the test drug was identified and authenticated by Siddha experts. The drug was prepared as per the procedure and subjected to various studies such as qualitative, quantitative, toxicity and pharmacological activities. Qualitative analysis includes Chemical analysis and Physico- chemical properties of *Pattai Vallathagi*.

Quantitative analysis included ICP-OES and HR SEM analysis to reveal its potency and effectiveness against the disease.

From the above analysis we came to know the presence of active ingredients responsible for its activity.

Literary collections:

Literary collections include drug review, which consist of both Botanical aspect, Gunapadam aspect, Pharmacological review that supported the study.

Chemical analysis:

Chemical analysis of the drug *Pattai Vallathagi* revealed the presence of Sulphate, Calcium, Iron,Photassium, tannic acid and alkaloid.

Calcium :

Skin disease may occur as a symptom of calcium deficiency.Presence of calcium in Pattai *Vallathagi* is useful in skin disease.

Pottasium :

Skin disease associated with nervousness may occur as a result of potassium deficiency.The existence of potassium in *Pattai Vallathagi*,So it is useful in skin disease.

Tannic acid[27]

The properties of tannic acid are anti-bacterial,anti-dermatoic anti septic properties.Thus it work well in skin disease

These chemical elements present in *Pattai Vallathagi* enhances the anti histamine activity of the drug.

The microbial load analysis confirms *Pattai Vallathagi* was free from microbial organisms and fungal infections.

In ICP-OES study, heavy metals were found below detection limit in *Pattai Vallathagi*. Calcium, Potassium, Phosporous, sodium, sulphur were present.

In HR SEM analysis, the particle size of *Pattai Vallathagi* was analyzed and reported. This ensures the absorption of the drug was more active and the drug have increased bio-availability.

Toxicological studies:

In acute oral toxicity study, there were no abnormal signs and behavioral changes in rats upto the dose level of 2000 mg/kg body weight administered orally. No mortality was observed in all groups. No abnormalities was seen in external observation and necropsy examination on the dose level of 5, 50,300 mg/kg b.w and 2000 mg/kg b.w. All the vital organs were normal.

In 28 days Repeated oral toxicity and 90 days Repeated oral toxicity study , the experimental animals were sacrificed by excessive anesthesia and blood samples were collected and sent for investigation. There were no significant changes in body weight, food and water intake, hematological parameters, renal parameters, Liver function test , Lipid profile and blood glucose level. The organs were collected and sent for histopathology study. It revealed the organs such as heart, kidney, liver, splean, brain was normal in Control, Mid dose and High dose. Thus the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

Pharmacological studies:

The pharmacological study was carried out in the animal model Wistar albino rats swiss albino mice and guinea pig. Three activities were seen in the drug *Pattai Vallathagi* The activities were

Anti- inflammatory

Analgesic

Anti -histamine

Anti- inflammatory activity

Administration of *Pattai Vallathagi* at the dose of 200 mg/kg and 400 mg/kg exhibited significant anti - inflammatory activity in Cotton pellet granuloma mothod.

Analgesic activity

Administration of Pattai Vallathagi at the dose of 200 mg/kg and 400 mg/kg exhibited significant analgesic activity in Eddy's Hot plate method.

Anti – histamine

In the present study the trial drug *Pattai Vallathagi* significantly inhibited the histamine induced contraction of isolated guinea pig ileum preparation,indicating Anti-histamine activity

From the discussion, it is concluded that the test drug *Pattai Vallathagi* is a safe and a potent anti- histamine drug. It also possess rich Analgesic and Anti inflammatory activity.

SUMMARY

10.SUMMARY

- The test drug ***Pattai Vallathagi*** was selected from the siddha literature ***"Siddha anuboga vaithiya navaneetha thirattu (part -10)"*** for its anti-inflammatory, analgesic and anti-histamine activities**.**

- The test drug was prepared by the given procedure. All the ingredients were identified and authenticated by the experts.

- Review of literature in various categories was carried out. Siddha aspect, botanical aspect disclosed about the drug and the disease.

- The drug was subjected to analysis such as physicochemical, phytochemical,biochemical and also instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug

- Toxicological study was made according to OECD guidelines comprising acute, sub-acute and sub chronic toxicity study. It screens the safety of the drug which attributes its utility in long time administration.

- Pharmacological study was done. It revealed the anti-inflammatory,analgesic and anti-histamine activities**.** and activities of ***Pattai Vallathagi*** in animal model Wistar albino rat,mice and guinea pig

- Results and discussion gives the proper justifications to prove the potency of the drug.

- Thus the herbao mineral formulation ***Pattai Vallathagi*** is validated for its safety and efficacy for treating skin diseases and it would be a great drug of choice.

CONCLUSION

11.CONCLUSION

From the literature evidence, Physico chemical analysis, chemical analysis, Toxicological evaluation and Pharmacological studies, the author concludes that the drug *Pattai vallathagi* is **safe** and it has significant effect in **anti-inflammatory, analgisic and anti - histamine activities** It is concluded that the drug *Pattai vallathagi* can be used in the management of skin diseases which is cost effective and easy to prepare.

ANNEXURE

12. ANNEXURE

The Tamil Nadu Dr.M.G.R. Medical University

#69, Anna salai, Guindy, Chennai-600 032.

This certificate is awarded to

Dr./~~Mr.~~/~~Ms.~~ A. ANBARASI

for participating as ~~Resource~~ ~~Person~~ / Delegate in the Fourteenth Workshop on

"Research Methodology & Biostatistics"

for AYUSH Post Graduates & Researchers

Organised by the Department of Siddha

The Tamil Nadu Dr. M.G.R. Medical University from 5th to 9th May 2014.

Dr. N. KABILAN M.D. (Siddha)
Reader, Dept. of Siddha

Dr. JHANSI CHARLES, M.D.
Registrar

Prof. Dr. D. SHANTHARAM, M.D., D.Diab.,
Vice-Chancellor

K.K. COLLEGE OF PHARMACY

(Approved by AICTE, PCI & Government of Tamilnadu and
Affiliated to The Tamilnadu Dr. MGR Medical University)

1/161, Sankaralinganar Road, • Gerugambakkam, • Chennai - 600128

Phone : (044) 32546162, Tele/Fax : 23821272

Ref: 4522/KKCP/2015 **Date: 10.08.2015**

APPROVAL CERTIFICATE

This is to certify that the project title *"Safety and pharmacological profile of **PATTAI VALLATHAGI**' has* been approved by IAEC and the details are furnished under

Project Code	Name of the species	Breakup sexwise	Weight	Number proposed	Number approved
KKCP/2015/026	Wistar Albino rat	25 Male + 31 female	150–200gms	60	56
	Swiss Albino mice	12 male + 12 female	20 – 25 gms	24	24
	Guinea pig	1 male	350– 400gms	4	1
Wistar Albino rat – fifty six ; Swiss Albino mice – twenty four; Guinea pig- one only Total number of animals -Eighty one only					

Chairman IAEC

(Prof. A. Meena)

CPCSEA Nominee

10/08/15

(Dr. C. Kathirvelan)

Veterinary Officer

(V. VAIDHYALINGAM)

Members

Dr. K. Sadasivan Pillai

10/8/15

CERTIFICATE

This is certify that the project title..... SAFETY PROFILE OF .....
..... "PATTAI VALLATHAGI" (12 Male + 12 Female rats) Wistar albino .....

has been approved by the IAEC(NO: NIS/IAEC-I/2016/01)

Name of Chairman/Member Secretary IAEC: nominee:

Dr. B.R SENTHILKUMAR

Signature with date

Name of CPCSEA

K. NACHIMUTHU

17/02/2016

Chairman/Member Secretary of IAEC:

24, FEB 2016

CPCSEA nominee:

(Kindly make sure that minutes of the meeting duly signed by all the participants are maintained by Office)

NATIONAL INSTITUTE OF SIDDHA, CHENNAI - 600047

BOTANICAL CERTIFICATE

Certified that the following plant drugs used in the Siddha formulation **"Pattai vallathagi"** (Internal) taken up for Post Graduation Dissertation studies by **Dr.A.Anbarasi**, M.D.(S), II year, Department of Gunapadam, 2015, are identified through Visual inspection, Experience, Education & Training, Organoleptic characters, Morphology, Micromorphology and Taxonomical methods as

Smilax china Linn. (Liliaceae), Root
Curcuma longa Linn. (Zingiberaceae), Finger rhizome
Semecarpus anacardium Linn.f. (Anacardiaceae), Nut
Sesamum indicum Linn. (Pedaliaceae), Seeds and Seed oil
Borassus flabellifer Linn. (Arecaceae), Palm jaggery

MEDICINAL BOTANY CHENNAI 600 047 of Siddha

Certificate No: NISMB1962015

Date: 14-8-2015

Authorized Signatory
Dr. D. ARAVIND, M.D.(s),M.Sc.,
Assistant Professor
Department of Medicinal Botany
National Institute of Siddha

சித்த மருத்துவ மைய ஆராய்ச்சி நிலையம், அரும்பாக்கம், சென்னை - 600106
सिद्ध केन्द्रीय अनुसंधान संस्थान, अरुम्बाक्कम, चेन्नै - 600106
Siddha Central Research Institute
(Central Council for Research in Siddha, Ministry of AYUSH, Govt. of India)
Arumbakkam, Chennai – 600106
[Ph: 044-26214925, 26214809, Fax: 26214809, Email: crisiddha@gmail.com, Web: www.siddhacouncil.com]

15.3.2016

CERTIFICATE

Certified that the samples submitted for identification by Dr. A. Anbarasi, III year MD Student, Department of Gunapadam, National Institute of Siddha, Sanatorium, Chennai-600 047 is identified as Ganthagam – Sulphur.

(R. Shakila)
Research Officer (Chemistry)

(Dr. P.Sathiyarajeswaran)
for Assistant Director (Scientist 2)-I/c

SOPHISTICATED ANALYTICAL INSTRUMENT FACILITY
INDIAN INSTITUTE OF TECHNOLOGY, MADRAS
Chennai - 600 036. INDIA

CERTIFICATE

This is to certify that Herbal/Mineral Drug **Pattai Vallathagi** formulated by **Dr.A.Anbarasi,** III year M.D(S), Department of GUNAPADAM, National Institute of Siddha, Chennai-47. Was analysed (qualitative/quantitative) by, SEM and ICPOES methods at SAIF, IITM, Chennai-36, during March 2016.

[DR.R.MURUGESAN]

Dr. R. Murugesan
Senior Scientific Officer
SAIF, IIT, Madras, Chennai-36.

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e-mail : saif@iitm.ac.in http://www.saif.iitm.ac.in

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